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THE ASCORBIC ACID CONTENT OF DIFFERENT VARIETIES OF MAINE-GROWN TOMATOES AND CABBAGES AS INFLUENCED BY LOCALITY, SEASON, AND STAGE OF MATURITY[1]

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INTRODUCTION

In 1935, studies of food production by Dove (*6*)[3] indicated that dietary vitamin C was lacking in various parts of Maine. The soundness of this observation has been confirmed in at least one section of the State by a clinical and dietary study of vitamin C nutrition in school children (*4, 14*).

The determination of the vitamin C content of Maine-grown foods has therefore become an important part of the experimental nutritional program of the Maine Agricultural Experiment Station (*15*). This program involves the investigation of many of the determining factors which affect the vitamin C content of fruits and vegetables. Of these factors, differences concomitant with variety are of extreme importance. The effects of maturation, distribution of the vitamin within the plant, soil type, fertilizer treatment, and geographical situation are also being observed.

Beacham and Bonney (*1*) found that Valencia oranges (from sour orange rootstock) grown in three localities in Florida varied in ascorbic acid content from 0.36 to 0.63 mg. per cubic centimeter of orange juice. The variations within each locality were nearly as large: in Orlando, from 0.44 to 0.63 mg. per cubic centimeter; in Mims, from 0.40 to 0.60 mg. per cubic centimeter; and in Wabasso, from 0.36 to 0.60 mg. per cubic centimeter. French and Abbott (*7*) found no correlation between the ascorbic acid content of oranges and the location in which they were grown. Metcalfe, Rehm, and Winters (*12*) observed inconsistent locality variations in oranges and grapefruit and attributed the differences to variations in soil.

To determine the effects of geographical situation on the production of ascorbic acid in plants, three experiments were carried out, one in 1938 and two in 1939.

[1] Received for publication April 17, 1941. Paper 237 of the biological laboratory of the Maine Agricultural Experiment Station.

[2] The author is deeply grateful to Dr. W. F. Dove for invaluable critical assistance in setting up this experiment and interpreting the results, to Arthur Hawkins and Iva M. Burgess for the supervision of experimental plots, and to Paul L. Johnson for technical assistance.

[3] Italic numbers in parentheses refer to Literature Cited, p. 501.

EXPERIMENT WITH TOMATOES, 1938

PROCEDURE

Four varieties of tomatoes (*Lycopersicum esculentum* Mill) were chosen to be grown in four widely different sections of the State of Maine. The localities selected were Aroostook Farm, at Presque Isle, in the northern part of the State; Orono in the central part; Highmoor Farm, at Monmouth, which is rather more southern; and Kennebunk, which is not far from the southern extremity of the State.

The varieties chosen were Penn State Earliana, Bestal, Best of All, and Comet. The two first named, from preliminary tests, appeared to be relatively low in ascorbic acid content; the other two were at the upper end of the scale. By selecting these extremes, any variations due to geographic situation within this large State would soon become apparent.

Because of the many factors that may occasion differences in ascorbic acid content, the data shown in table 1 were recorded for the four localities in which the plants were grown.

The plants were started from seeds which were planted in sterile sand April 23, 1938. On May 16 the seedlings were transferred into bands in plots of soil. The young plants were set outdoors on the following dates: At Aroostook on June 17; Orono, June 4; Highmoor, June 10; and Kennebunk, June 3.

Testing was begun August 11, and the tomatoes were analyzed at weekly intervals thereafter for a period of 6 weeks. The plants grew and fruited at different rates in the different localities. The weather conditions during the summer of 1938 were not auspicious for tomato ripening in Aroostook, but in the southern sections they were particularly conducive to good growth and development. This fact made for a diversity among the samples that was undesirable. Thus, although conditions were controlled as completely as possible, the samples were not strictly comparable at all times.

TABLE 1.—*Soil type, latitude, and length of growing season at the 4 localities in which tomatoes were grown*

Locality	Soil type	Latitude	Length of growing season (*11*)
			Days
Aroostook	Caribou loam	46°40′30″	90–110
Orono	Silty clay loam	44°54′2″	120–130
Highmoor	Heavy loam	44°14′24″	140–150
Kennebunk	Adams sandy loam and fine sandy loam.	43°24′	160–170

Even green tomatoes were not available from Aroostook at the first testing date. At Highmoor and Kennebunk, the fruit matured early and samples were not obtainable for the later testing dates. Not more than 10 ripe tomatoes were harvested in Aroostook, making it necessary to compare green with ripe tomatoes after the third testing date. Through an oversight, the Bestal variety was not included at Highmoor so that the data for that section were not complete.

The material to be tested was harvested in each of the four sections on the same day at approximately the same time. The analyses were made at Orono. The tomatoes were sent by express from the other localities and arrived usually about 12 hours after they were harvested. The Orono samples were kept at room temperature until the other samples arrived. All the samples were analyzed at the same time.

A composite sample to weigh between 20 and 30 gm. was made up of radial sectors from each of four tomatoes. Duplicate samples consisted of the opposite radial sectors from the same tomatoes. The ascorbic acid content was determined by the titrimetric method developed by Bessey and King (*3*) as later modified by Musulin and King (*16*). Three percent metaphosphoric acid was used as an extractant according to recommendations by Bessey (*2*). The results of the analyses are presented in figure 1.

EFFECT OF LOCALITY

It may be seen that, with the exception of one variety, all the tomatoes from Aroostook were lower in ascorbic acid content than those from the other localities. The Aroostook-grown Bestal tomatoes manifested higher ascorbic acid values than those from Orono for 2 weeks. Perhaps it is significant that at the testing dates when the Aroostook Bestal tomatoes were at the highest peak, all the sample tomatoes of that variety were ripe. Maclinn, Fellers, and Buck (*9*) found no consistent increase in the vitamin C content of tomatoes during three stages of maturity. In order to determine the effect of ripeness at Orono, 10 pairs of samples of tomatoes including 7 varieties were analyzed. Both green and ripe tomatoes from the same plants were tested on the same day. In every case the ripe tomatoes were higher in ascorbic acid than the green. The mean difference was 0.09 ± 0.01 mg. per gram with infinite odds that the difference was significant.

The variations depicted in figure 1 may have been due to a number of influences. Although there is a difference of over 3° in latitude between Aroostook and Kennebunk, this seems not to have been the factor that caused the vitamin C differences since there is half that latitude difference between Orono and Kennebunk and the vitamin C values show no variations.

Killing frosts are common much later in the spring at Aroostook than at the other three localities. This necessitates setting plants out at a later date and, in consequence, the tissue tested at any given time is less mature than that from plants grown at the more southern localities. Although it has been shown that the green tomatoes from a given variety have lower ascorbic acid values than the ripe fruit, it is obvious from the graphs that the differences observed during this experiment were not due entirely to the immaturity of the tissue. At the first three testing periods all the tomatoes were green and the smaller content of ascorbic acid in the Aroostook samples was even then apparent.

Data on weather conditions for the four localities under investigation were incomplete. For Kennebunk, it was necessary to use the data accumulated at Portland, Maine, which is approximately 30 miles northeast of Kennebunk. Both, however, are coastal towns and weather conditions are similar. For Highmoor, the data accumulated

at Lewiston (12 miles southwest) were used. For Orono and Aroostook the weather data were recorded at the locality in which the crops were grown.

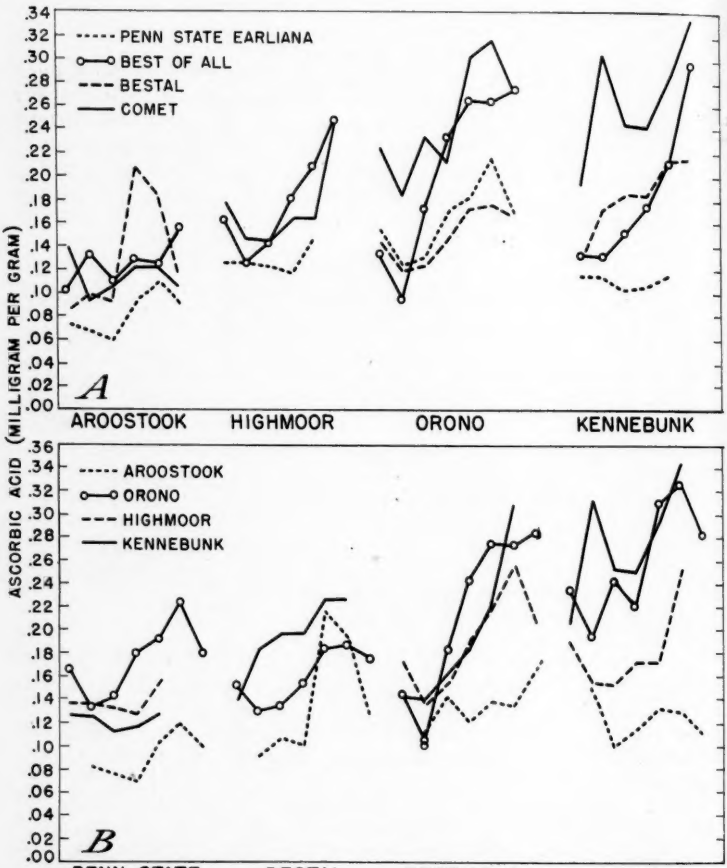

FIGURE 1.—The ascorbic acid content of tomato fruit grown in four different localities in Maine during the summer of 1938: *A*, Amounts of ascorbic acid arranged according to locality in which tomatoes were grown; *B*, amounts arranged according to variety. Determinations were made at weekly intervals and are shown in chronological order.

The average temperature during the growing season of 1938 in Aroostook was somewhat lower than in the other places: for Aroostook 60.6° F.; for Orono, 66.8°; for Highmoor, 68.0°; and for Kennebunk, 66.6°. It seems unlikely that these differences were large enough to

be the chief factor causing the ascorbic acid variations. If the temperature had been low enough to inhibit metabolic processes, the view that low temperature was responsible for low ascorbic acid would be more tenable.

EFFECT OF VARIETY

Varietal differences (fig. 1) were not as consistent as preliminary experiments had indicated. Originally fairly large differences were apparent between the Comet and the Penn State Earliana varieties when the plants were grown outdoors. The differences were smaller when the plants were grown in the greenhouse. The reverse appeared to be true of the Best of All and the Bestal varieties. The differences between these two were large when they were grown indoors, but smaller when they were grown outdoors.[4]

In this experiment the Penn State Earliana tomatoes exhibited the smallest values for vitamin C of the four varieties in all sections except Orono, where it showed a larger average value than Bestal. Bestal was the most variable of the varieties in respect to vitamin C, its values ranging from 0.09 to 0.21 mg. per gram in Aroostook alone. In Aroostook, the Bestal had the highest average value (0.14 mg. per gram) of the four varieties. The average values for Bestal and Best of All were exactly the same (0.20 mg. per gram) at Kennebunk. Bestal showed a lower value than Comet and Best of All at Orono.

The foregoing data suggested a trend which called for further investigation to determine whether vitamin C values are consistently on a lower level in Aroostook or whether the data designated a spurious phenomenon peculiar to the season of 1938. Should the former prove to be the case, it would be necessary to obtain varieties which are early, adaptable, and rich in vitamin C when grown locally to provide this large northern section of the State with at least a part of its supply of this essential vitamin.

EXPERIMENT WITH TOMATOES, 1939

In order to increase the reliability of the observations made during the season of 1938 on differences in the vitamin C content of tomatoes coincident with geographical situation, the experiment was repeated during the summer of 1939.

Although no ripe tomatoes had been available from Aroostook during the 1938 testing season, it was deemed advisable to adhere to the experimental procedure as originally planned rather than to add new variables by changing the varieties to be observed.

PROCEDURE

The plants were set out on the same locations as were used in 1938 so that the data recorded above as regards soil type, latitude, and length of growing season are applicable to the second season's experimentation. The soils for the four plots were analyzed and the data are recorded in table 2.[5] The young plants were set outdoors on the

[4] McINTOSH, J. SOME FACTORS AFFECTING THE VITAMIN C CONTENT OF TOMATOES AND RUTABAGAS. Thesis Univ. of Maine. 1938.

[5] Grateful acknowledgment is made to Dr. D. S. Fink for the analyses of soils.

following dates: At Aroostook, June 14, 1939; Orono, June 6 and 7; Highmoor, June 8; and Kennebunk, June 6.

Testing was begun on August 10, approximately the same date as in the previous season. Subsequent determinations were made weekly until September 21. Cultural conditions were similar to those of the preceding year. The method of sampling was identical with that outlined for the 1938 experiment.

TABLE 2.—*Analytic data for soils in the experimental plots at the four localities in which tomatoes were grown in 1939*

Locality	Reaction (pH)	Nitrates	Ammonia	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron	Aluminum
Aroostook	[1] 6.29	[2] 100	[3] 0+	[4] 50+	[5] 100	([6])	[2] 400	[5] 25−	[3] 5−	.....
Orono	6.00	([6])	0+	200	([6])	2,000	100+	10−	5+	250−
Highmoor	5.67	([6])	400	200−	([6])	1,500	100−	10−	5	250−
Kennebunk	5.01	([6])	200−	300	([6])	3,000	200	25+	5	100

[1] Moderate.
[2] Very high.
[3] Trace.
[4] Medium low.
[5] Low.
[6] Excess.

EFFECT OF LOCALITY

At the end of three testing periods it was obvious that history was repeating itself only insofar as to show that during any one season, locality had a marked effect upon ascorbic acid production. During these three periods, the tomatoes from Aroostook were found to contain nearly as much ascorbic acid as those from Orono, while the samples from the two more southern localities contained much less than those from Orono and Aroostook. The amounts of ascorbic acid in the samples for the entire period are shown graphically in figure 2.

Soil type and fertilizer treatment have been reported as playing a part in the production of ascorbic acid by plants (*19*), although later observations do not confirm this finding (*10*).

Soil analyses were not made during the 1938 investigation. The soil analytic data for the 1939 experiment were examined on the presumption that differences in available soil nutrients may have caused the variations in ascorbic acid content. Insofar as soil analyses reveal the quality of the soil as regards those elements now known to be essential, it was judged that neither soil type nor available nutrients were responsible for the ascorbic acid variations in this experiment. This does not preclude the possibility that the soil condition may be found to be a determining factor in vitamin C synthesis in the light of future advances in soil science.

The possibility of a loss through oxidation of the ascorbic acid between the times of harvesting and testing was suggested. Although the tomatoes were harvested at approximately the same times and held at room temperature until analysis was begun, there was a possibility that shipping conditions from Kennebunk and Highmoor were of such a nature as to destroy vitamin C. High temperatures would be the main condition that might bring about this destruction. Although Maclinn, Fellers, and Buck (*9*) found no diminution in the ascorbic acid content of tomatoes stored at room temperature up to

240 hours, it was possible that the experimental conditions in Maine were not exactly analogous to those in Massachusetts.

Accordingly it was arranged to have the tomatoes shipped in containers with solid carbon dioxide whereby the temperatures were kept near or below freezing. The samples were shipped in this way for three testing periods. The ascorbic acid values of these tomatoes are recorded in table 3.

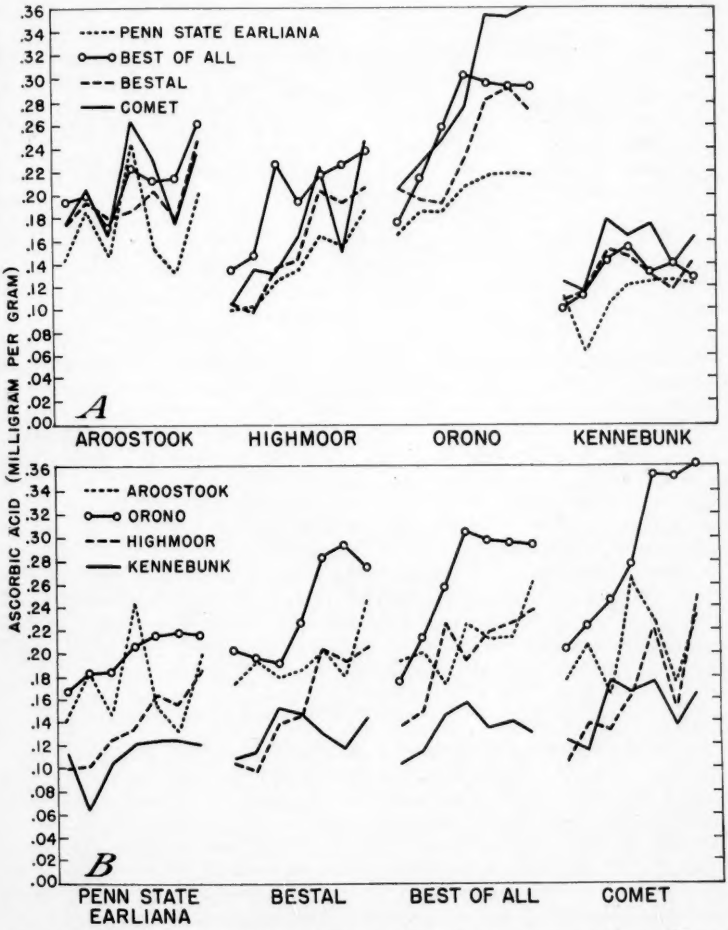

FIGURE 2.—The ascorbic acid content of tomato fruit grown in four different localities in Maine during the summer of 1939: *A*, amounts of ascorbic acid arranged according to locality in which tomatoes were grown; *B*, amounts arranged according to variety. Determinations were made at weekly intervals and are shown in chronological order.

TABLE 3.—*Effect of low temperatures during shipping on the ascorbic acid content of tomatoes*

[Four varieties grown at 4 localities in Maine]

Variety	Number of analyses and shipping conditions	Aroostook	Orono	Highmoor	Kennebunk
		Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
Penn State Earliana	3 analyses; with solid carbon dioxide.	0.18	0.21	0.15	0.12
	4 analyses; without solid carbon dioxide.	.17	.19	.13	.10
	7 analyses; with and without solid carbon dioxide.	.17	.20	.14	.11
Comet	3 analyses; with solid carbon dioxide.	.22	.33	.18	.16
	4 analyses; without solid carbon dioxide.	.20	.26	.16	.15
	7 analyses; with and without solid carbon dioxide.	.21	.29	.16	.15
Bestal	3 analyses; with solid carbon dioxide.	.19	.27	.18	.13
	analyses; without solid carbon dioxide.	.20	.22	.14	.13
	7 analyses; with and without solid carbon dioxide.	.19	.24	.16	.13
Best of All	3 analyses; with solid carbon dioxide.	.22	.30	.21	.14
	4 analyses; without solid carbon dioxide.	.21	.24	.19	.12
	7 analyses; with and without solid carbon dioxide.	.21	.26	.20	.13

It will be noted that the figures for Kennebunk and Highmoor are proportionately the same when compared to those for Aroostook and Orono as they were before the precaution of icing was taken. They are comparable to the values obtained while the samples were shipped at the existing temperatures or to the average of all the determinations including those from fruits shipped with and without ice. The slightly higher figures obtained for tomatoes shipped in ice were caused by the fact that during these periods practically all of the tomatoes were ripe, whereas two or three of the four samples averaged for the groups shipped without ice consisted of green tomatoes. Hence an effect of maturity is manifested in these higher values. Loss of vitamin content through oxidation caused by high temperatures apparently was not the cause of the locality variations. The tomatoes analyzed on the last testing date were not shipped in ice.

EFFECT OF VARIETY

Varietal differences were similar to though less variable than those observed in 1938. The data are shown in figure 2. In all four localities the Penn State Earliana variety had the lowest values for ascorbic acid content. Bestal was slightly higher than Earliana in all localities and was consistently the second lowest. Comet was highest at Orono and Kennebunk, whereas Best of All was highest at Aroostook and Highmoor. The values for these two latter varieties were quite similar so far as variety is concerned, although variations within variety caused by geographical situation were comparatively large.

EFFECT OF MATURITY

Again, in the season of 1939 increases in ascorbic acid coincident with maturation were evident. A gradual rise with the advent of

ripeness was noted, with more or less constant values apparent after complete ripeness was attained. The effect of overripeness was not determined. The averages for the green and the ripe tomatoes are listed in table 4. It is interesting to note that while five of the seven samples tested from Aroostook consisted of green tissue, the values are still higher than those of Highmoor and Kennebunk where five of the seven samples were ripe tomatoes. Locality differences are large enough to overshadow the effects of maturity.

EFFECT OF WEATHER CONDITIONS

Weather conditions were better in Aroostook in 1939 than in 1938. In spite of this, ripe tomatoes were available for only three of the testing dates, whereas five samples of ripe fruit were available from the other localities. At Kennebunk and Highmoor, there were few clear sunny days. Especially at Kennebunk, heavy fog prevailed over long periods. Excellent fruit was shipped in, however, from the two southern localities, and the local growers considered 1939 to be a good tomato season.

The available weather data were inadequate to establish a premise on the basis of meteorological facts. The amount of sunshine is not recorded in Orono or Aroostook except in terms of clear, cloudy, and partly cloudy days. It was possible to calculate the hours of sunshine from the Lewiston records, and the percentage of possible sunshine was recorded at Portland. Temperature and rainfall data were accurately recorded in all four localities.

TABLE 4.—*Effect of maturity on ascorbic acid content of 4 varieties of tomatoes grown at 4 different localities in Maine in 1939*

Variety	Condition of fruit	Ascorbic acid content of tomatoes from—			
		Aroostook	Orono	Highmoor	Kennebunk
		Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
Penn State Earliana	Green	0.15	0.18	0.10	0.09
	Ripe	.22	.21	.15	.12
Comet	Green	.19	.21	.12	.12
	Ripe	.25	.32	.18	.17
Bestal	Green	.19	.20	.11	.11
	Ripe	.20	.25	.19	.14
Best of All	Green	.19	.19	.14	.12
	Ripe	.23	.29	.22	.14

It has been shown by Kohman and Porter (*8*) that the solar rays impinging upon tomato plants immediately before testing greatly affect the ascorbic acid content. These workers stated that when tomato plants were taken from the outdoor sunshine into the greenhouse, a marked drop in vitamin C resulted. Reid and Weintraub (*18*) and Reid (*17*) observed an increased synthesis of ascorbic acid when excised moonflower roots and young cowpea plants were grown in light instead of darkness. Currence (*5*) found no significant correlation between the vitamin C of tomato plants and the percentage of sunshine. His plants were grown in a greenhouse, however, where environmental conditions are not comparable to those of the outdoors (*8*). Furthermore, all of his reported values were relatively low, ranging from 0.09 to 0.14 mg. per gram. Varieties with high

vitamin C values may emphasize a relationship to light if one exists. Currence's data were taken during November, December, and January when even a high percentage of possible sunshine may not have been favorable for maximum vitamin C production. Reid [6] noted that roots with a low content of ascorbic acid during a prolonged cloudy period showed a marked increase of this substance following a day of bright sunshine. Moldtmann (*13*) pointed out that a lowering of the vitamin C content occurs also in the leaves of *Fagus sylvatica* during periods of cloudy weather, while an increase is apparent during sunny weather. These observations indicate that the environmental conditions to which the plant is exposed immediately prior to analysis have a disproportionate influence on ascorbic acid synthesis in relation to conditions which exist during a complete season. Therefore, averages of weather data were calculated for the growing season (from planting date until date of last harvest), for the testing season, for 3 days before each harvest, and for the day of harvest. The available data are presented in table 5.

TABLE 5.—*Temperature, rainfall, and sunshine at the four Maine localities in which tomatoes were grown during the summers of 1938 and 1939*

Locality	Year	Average temperature		Rainfall				Sunshine			
								Total, 3 days before harvest		Total, day of harvest	
		Growing season [1]	Testing season [1]	Growing season [1]	Testing season [1]	Total, 3 days before harvest	Total, day of harvest	Hours of sunshine [2]	Percent of possible sunshine	Hours of sunshine [2]	Percent of possible sunshine
		F.	*F.*	*Inches*	*Inches*	*Inches*	*Inch*	*Hours*	*Percent*	*Hours*	*Percent*
Aroostook	1938	60.6	56.1	15.72	6.49	2.57	0.70				
	1939	64.4	63.8	13.80	4.32	2.05	.96				
Orono	1938	66.8	63.2	11.84	5.23	1.12	.76				
	1939	66.2	65.8	9.60	2.22	2.45	.24				
Highmoor [3]	1938	68.0	63.2	15.00	5.33	1.84	.12	159.5		45.5	
	1939	68.1	68.1	9.60	1.63	3.07	.43	117.2		44.0	
Kennebunk [4]	1938	66.6	67.9	17.40	2.06	.03	.01		91		75
	1939	65.9	66.5	11.31	2.49	2.95	.37		57		70

[1] The growing season was from planting date to date of last harvest; the testing season from first to last harvest.
[2] The writer is indebted to Paul A. Bean, of Lewiston, Maine, for the sunshine records.
[3] Lewiston.
[4] Portland.

An analysis of the data shows that at Aroostook in 1938, when vitamin C values were low, rainfall during the growing season amounted to 15.72 inches, whereas in 1939, when vitamin C values were higher, rainfall was 13.80 inches. For the 43 days of the testing period, the 1938 rainfall was 6.49 inches and the 1939 rainfall 4.32 inches—a decrease of 33 percent. Less difference was observed between the averages of the 3 days before harvests, and the 1939 rainfall was higher when harvest dates alone are considered. The mean temperature was 3.8° lower in 1938 during the growing period and 7.7° lower during the testing period. There were 12 clear days and 28 cloudy

[6] REID, MARY ELIZABETH. Personal communication, 1941.

or partly cloudy ones during the 1938 testing period as compared with 17 clear days and 25 cloudy ones during 1939.

Orono conditions were similar for both seasons with somewhat less rainfall in 1939.

At Highmoor and Kennebunk the rainfall was less in 1939 during both growing and testing periods. In 1939 there was about double the amount of rainfall at Highmoor when the 3-day periods prior to harvest were totaled and at Kennebunk the increase was very large.

The records for available sunshine were fairly adequate for Lewiston and Portland, and these tend to uphold the premise that sunshine is one of the chief factors contributing to the production of ascorbic acid by the plant. The variations are striking during the 3-day periods.

EXPERIMENTS WITH CABBAGE, 1939

To obviate drawing conclusions from results limited to one vegetable whose reactions to environmental factors might possibly be unique, an analogous experiment with another crop was planned and executed in 1939. Cabbage (*Brassica oleracea L.* var. *capitata L.*) was chosen for this parallel experiment, since it can be grown to maturity in all parts of Maine and is known to be an excellent source of vitamin C.

Four varieties, each representing a type, were planted in adjacent plots in the four localities. Soil analytic data were similar to those for the tomato plots. The varieties planted were as follows:

Variety	*Type represented*
Cornell Early Savoy	Savoy.
Yellows Resistant Copenhagen	Copenhagen Market.
Jersey Wakefield	Wakefield.
Penn State Ballhead	Danish Ballhead.

The first three varieties mature during the latter part of July and the first part of August. The Penn State Ballhead is in its prime about the first of October, when the other varieties are either unavailable or, at least, are definitely past the peak of their development.

The cabbage seed was treated with Semesan and planted in sand on May 2, 1939, and the young seedlings were transferred to soil on May 18. The plants were transported to the field and set out on the following dates: At Aroostook on June 14; Orono, June 6 and 7; Highmoor, June 15; and Kennebunk, June 16.

Once weekly for 3 weeks the young plants were treated with mercuric chloride solution to prevent maggot injury.

Because of the differences in time necessary to attain maturity, testing was started early in the season and carried out through October when the Ballhead variety became mature. The plants were shipped by express to the Orono laboratory and were analyzed immediately upon arrival. Similar conditions of harvesting and shipping were maintained with the cabbages as with the tomatoes, including the interval when solid carbon dioxide was used to control the temperature during shipment. No losses ascribable to shipping conditions were observed.

Testing was begun July 19, 1939, and subsequent determinations were made at biweekly intervals until October 11. During July, the plants were large but the heads were just beginning to form. The plants from Aroostook were smaller than those from the more southern

localities. During the latter part of the season, the earlier varieties were not available at all of the localities. Those that could be procured are included in the report if at least two places are represented.

The sampling procedure presented difficulties. It was desired to keep the samples relatively small so that dissolution of the vitamin in the extractant would be complete. On the other hand, differences in vitamin C values among analogous cabbages as well as uneven distribution of the vitamin within individual plants made it imperative to use several vegetables for a composite sample in order to have the sample truly representative of the variety.

A very thin cross section was taken through the whole cabbage, cutting as near as possible at the center of the head. When the plants were young and immature, this region was very near the base stalk. As the head grew larger, the sample was taken farther away from the base. None of the hard main stalk was included in the sample, but

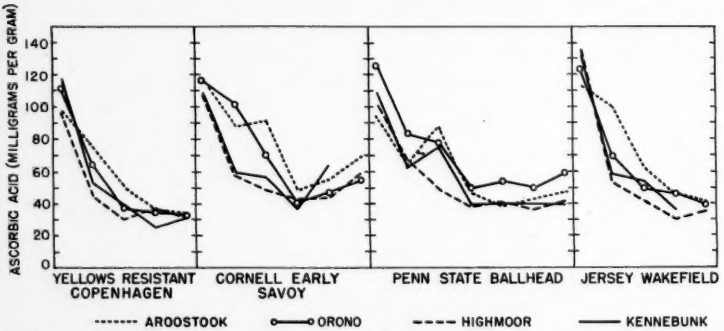

FIGURE 3.—The ascorbic acid content of four varieties of cabbage grown in four different localities in Maine during the summer of 1939. Determinations were made at biweekly intervals and are shown in chronological order.

the smaller stalks of the large, individual outer leaves were sectioned along with the rest of the cabbage. Composite samples consisted of these very thin sections from three plants whenever possible. As a result of excessively heavy rains in Aroostook some of the plants were washed away, leaving a shortage of test material. Therefore, it was necessary occasionally to use two plants and sometimes only one plant for a sample. Duplicate samples consisted of thin slices taken just above the original samples.

The results of the analyses are shown graphically in figure 3. The effects of maturity are worthy of note. When the plants were young and the tissue most highly active metabolically, the ascorbic acid values were extremely high, but with the approach of maturity they dropped rapidly. This condition is consonant with the view that vitamin C has a role to play in metabolism or is a byproduct thereof. The same diminution in ascorbic acid with the onset of maturity has been observed in onions and apples.[7] The converse seems to be true of tomatoes, i. e., as maturity is achieved, the ascorbic acid content increases. The sharp break in the curves demonstrating the values

[7] MURPHY, E. F. Unpublished data, 1940.

of Cornell Early Savoy cabbage at the end of the sixth week and a subsequent upward swing are worthy of note. A lack of test material prevented further investigation of this increase.

With but slight variations, all four varieties (fig. 3) show remarkable similarities in respect to vitamin content. A distinct trend in ascorbic acid decline as the plants approached maturity is clearly apparent for all four varieties in all four localities. Although the values are not plotted on a semilogarithmic scale, the manner in which the varietal lines bunch together on the arithmetic scale with narrow intervals indicates that the ascorbic acid per gram of cabbage of the four varieties not only decreases by similar absolute amounts with time but, as indicated by the similar slopes of the lines, decreases at approximately the same rate. The slight differences in rate of diminution for the different localities is not enough to erase the more favorable results in Aroostook and Orono. The rapid rate of decline, indicated by the steep slopes of the lines based on the earlier tests, could very well intensify the differences in favor of Aroostook and Orono because of the relative physiological immaturity of the plants from these regions. This variable would lose its importance, however, as all the plants become mature and all the curves level off, or even, as in the case of the Cornell Early Savoy, actually turn up. This variable might be eliminated from the charts by shifting the Orono and Aroostook curves to the left, but this would necessitate an arbitrary assumption as to the physiological age of the tissue. It was thought best not to do this, but caution is urged against a spurious interpretation from the middle sections of the charts. The leveled sections of the charts, when physiological age differences are presumably minimized, show a higher vitamin content in Aroostook and Orono in the majority of cases, and this is in essential agreement with the results of the tomato experiments discussed above.

DISCUSSION

The variations in ascorbic acid noted in food crops grown in different locations are shown here to be undoubtedly caused by environmental agents and therefore are seasonal rather than locality variations. To demonstrate the effects observed, the differences between each locality and every other locality were plotted. Two of these graphs are included. Figure 4 shows the variations in the vitamin C content of tomatoes grown in Aroostook as compared with Orono, Highmoor, and Kennebunk in 1938 and 1939. The dissimilarities of the charts for the 2 years are striking. In 1938 the ascorbic acid of Aroostook tomatoes was lower at all testing periods than that of tomatoes grown in the other three localities. In 1939 the values for Aroostook tomatoes were lower than those from Orono, somewhat higher than those from Highmoor, and markedly higher than those from Kennebunk. That this is a seasonal effect is apparent from figure 5, in which the differences between varieties in each locality for 1939 and 1938 are compared.

The differences between the ascorbic acid values of cabbages grown in Aroostook in 1939 and the same varieties grown in the other three localities are depicted in figure 6. The Aroostook-grown cabbage shows a higher ascorbic acid content per gram than those produced

in Orono, Highmoor, and Kennebunk for all varieties and for practically all testing periods except the first. It may be that the production of vitamin C increases from the time the plants are set out until the cells are no longer highly active in a metabolic sense and are substituting growth in size for cell division. If this assumption is correct, the peak may well have occurred in cabbages at the first testing date in Orono, Highmoor, and Kennebunk, whereas it may not have been attained in Aroostook until 2 weeks later, when the shift in the opposite direction is very marked. Thus a developmental may be superimposed upon a seasonal influence to increase the complexity of interpretation.

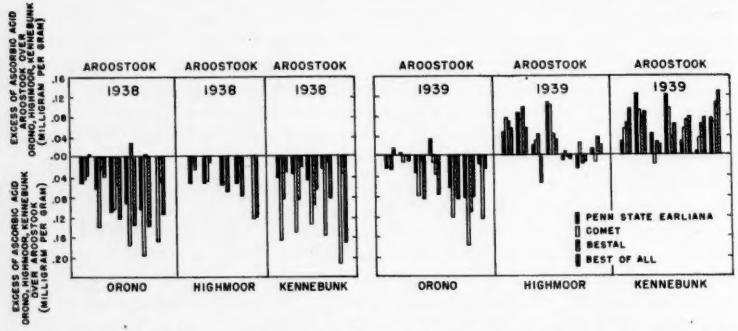

FIGURE 4.—The differences between the ascorbic acid values of four varieties of tomato grown in Aroostook and the same varieties grown in three other Maine localities. Each rectangle represents the ascorbic acid difference between tomatoes of one variety grown in two localities. Each group of rectangles represents one testing period, and the groups are arranged in chronological order of testing periods. Zero is an assumed point of uniformity at which there would be no locality variations.

TABLE 6.—*Variations in the ascorbic acid content of tomatoes as affected by environment and variety*

Successive weekly tests during fruiting period [1]	Environmental differences	Varietal differences	
	Average differences in vitamin C between the same varieties grown in 4 localities	Average differences in vitamin C between unlike varieties in each of 4 localities	Difference in favor of environmental effect
	Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*
1	0.037±.0064	0.023±.0052	0.014
2	.052±.0065	.035±.0058	.017
3	.041±.0048	.037±.0036	.004
4	.060±.0065	.034±.0040	.026
5	.067±.0071	.044±.0051	.023
6	.084±.0091	.035±.0035	.049
7	.077±.0082	.045±.0087	.032
Mean deviation for 7 periods	.060±.0029	.036±.0018	.024±.0034
Standard deviation	.0383	.0246	

[1] $N=12$ comparisons (4 varieties or 4 localities taken 2 at a time).

In table 6 the variations imposed by environment are compared to those inherent in the plants. The environmental difference shown for each testing period is the average of the differences in ascorbic acid

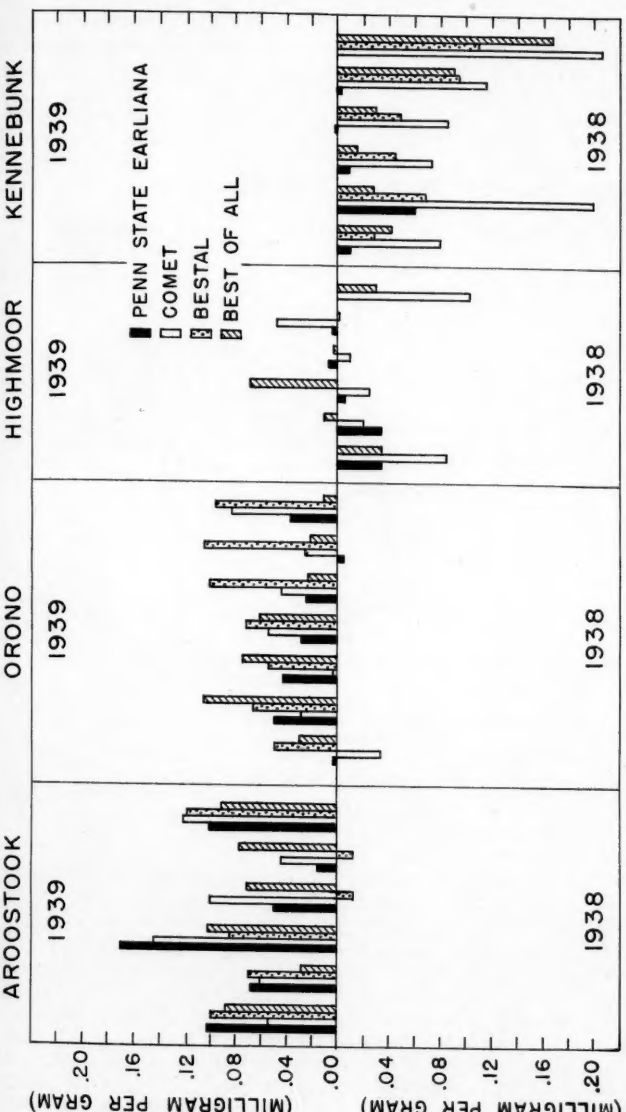

FIGURE 5.—Seasonal deviations in the ascorbic acid content of four varieties of tomato grown in four localities in Maine. The tomatoes were produced on the same plots each year. Each rectangle represents the ascorbic acid difference between 1939 and 1938 for one variety of tomato. Each group of four rectangles is arranged in chronological order of testing periods.

content between the values for the same varieties in four localities. The varietal difference for each testing period is the average of the differences in ascorbic acid content between the values in the same localities of four varieties. In the fourth column the values expressing the effect of environment are shown to be in excess of those expressing the effect of variety in all seven testing periods.

Currence (*5*) points out that environmental effects vitiate the significance of varietal difference, and that recommendations to growers emphasizing the nutritional superiority of any particular variety are misleading. In these experiments, the amounts of ascorbic

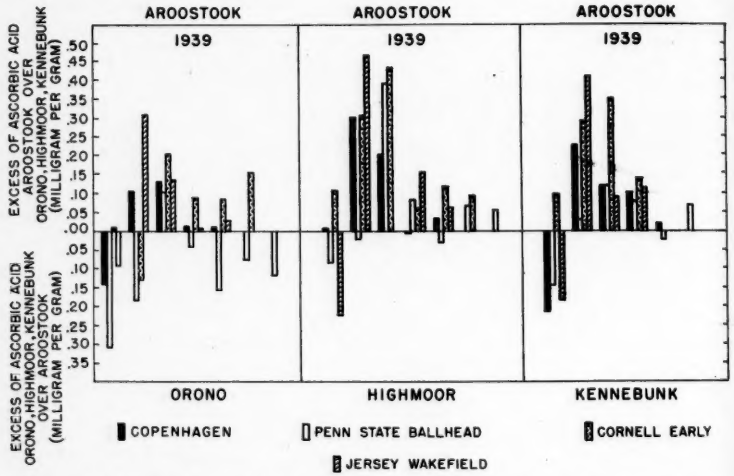

FIGURE 6.—The differences between the ascorbic acid values of four varieties of cabbage grown in Aroostook and the same varieties grown in three other Maine localities. Each rectangle represents the ascorbic acid difference between cabbages of one variety grown in two localities. Each group of rectangles represents one testing period, and the groups are arranged in chronological order of testing periods. Zero is an assumed point of uniformity at which there would be no locality variations.

acid coincident with variety as depicted in figures 1 and 2 are worthy of note. In spite of the magnitude of the variations caused by environmental agencies, varieties having high ascorbic acid values remain in a relatively high position even when grown under adverse environmental conditions, e. g., the Comet (a high variety) is significantly higher than Penn State Earliana (a low variety) in Kennebunk during 1939, although it is lower than Penn State Earliana grown in Aroostook in the same season, whereas Comet in Aroostook is significantly higher than Earliana in that locality. The mean differences of these comparisons are as follows:

Variety and locality, 1939		Mean difference A over B	
A	B	*Mg. per gm.*	Odds
Comet, Kennebunk, vs. Earliana, Kennebunk		$+0.0417 \pm 0.0054$	630 to 1
Comet, Aroostook, vs. Earliana, Aroostook		$+0.0346 \pm 0.0048$	499 to 1
Earliana, Aroostook, vs. Comet, Kennebunk		$+0.0201 \pm 0.0066$	17 to 1

Analogous findings during 1938 showed the Comet variety to be vastly superior to the Penn State Earliana. Some of the mean differences may be set forth as follows:

Variety and locality, 1938		Mean difference A over B	
A	B	*Mg. per gm.*	Odds
Comet, Aroostook, vs. Earliana, Aroostook		$+0.0325 \pm 0.0053$	158 to 1
Comet, Kennebunk, vs. Earliana, Kennebunk		$+0.1422 \pm 0.0113$	1361 to 1
Comet, Aroostook, vs. Earliana, Kennebunk		$+0.0043 \pm 0.0044$	2 to 1

The environment may exert an influence of such intensity as to cause a genetically high variety to produce less vitamin C than a genetically low variety. The third case shown in each of the two preceding summaries of mean differences reflects a trend typical of the reactions observed in this study. When 2 years' data from Aroostook and Kennebunk are analyzed together, the seasonal variations practically disappear, because Aroostook produced low- and Kennebunk produced high-vitamin-C tomatoes in 1938, and the opposite was true in 1939. The values were slightly more favorable in Kennebunk over the 2-year period, with a mean difference of 0.0373 ± 0.0221 mg. per gm. with odds of 6 to 1 in favor of Kennebunk.

Similar varieties from different localities show differences dependent upon the environmental conditions prevalent in each locality during the season. To illustrate this point, a few of the mean differences are set forth:

Variety and locality		Mean difference A over B	
A	B		
1938		*Mg per gm.*	Odds
Earliana, Aroostook, vs. Earliana, Kennebunk		$+0.0564 \pm 0.0007$	Infinite
Comet, Aroostook, vs. Comet, Kennebunk		$+0.1660 \pm 0.0081$	Infinite
1939			
Earliana, Kennebunk, vs. Earliana, Aroostook		$+0.0618 \pm 0.0108$	168 to 1
Comet, Kennebunk, vs. Comet, Aroostook		$+0.0547 \pm 0.0088$	240 to 1

Thus in comparing varieties within a rather limited locality, genes are found to exert a consistently significant influence. When the region is widened, e. g., into a State as large as Maine or even, as in this experiment, into areas delimited to less than one-half of the State, the influence of environment is manifested and apparently tends to neutralize the effect of genes, although this is purely a spurious phenomenon. In contrast, gene influence has an apparent neutralizing effect upon the environment. The genetic characteristic which effectively maintains high vitamin C constitutes a directive in the approach to the problem of increased vitamin C production for populations. Hence to ignore the importance of inherent qualities in respect to vitamin C would be an unwise omission. A variety nutritionally superior by virtue of its genes is proportionately superior even when subjected to an unfavorable environment.

The foregoing data give ample testimony to the doubtful nature of any one set of figures for the vitamin C content of a given fruit or vegetable. The necessity for using extreme care in evaluating diets and in assaying the available nutrients for specific crops or regions is manifest. It seems that observations covering several seasons' work on plants grown in widely separated localities would be more indicative of a truly genuine average value than would short-term experiments

of a few weeks' duration on localized samples. The results emphasize the importance of variety tests for more than one season on plants grown in the localities for which the varieties are intended.

The many known factors which play a part in the production of vitamin C during plant growth and in the retention of this food essential during storage, shipping, and cooking, as well as the less known influences which are gradually being brought to light, necessitate truly fine discrimination in utilizing the figures available in the literature. To achieve even a moderate degree of precision in the well-known types of vitamin C study and assay, it is apparent that an essential part of the experimental procedure would be direct analysis of the foods available in the region, whether locally grown, influenced as they are by seasonal factors, or of nonlocal origin, with consequent storage and shipping losses.

In view of the observations contained herein, it is clear that added to the influences imposed by maturity, variety, and metabolic rate, are those really large variations ascribable to seasonal influences.

The cause of these variations is uncertain. The writer hesitates to attribute the fluctuations in vitamin C values to the factors discussed above without more confirming evidence, but certain evidence indicates that preponderance of cloudy days, excessive rainfall, and low temperatures may well exert an unfavorable influence upon vitamin C synthesis.

In the experimental program outlined for this State, a slowness of approach is imposed by the very factors which come to light during the investigation of each unit. A survey of the vitamin C values of foods in so large a State requires adequate sampling within varieties as well as investigations along such lines as are discussed above. The ultimate aim of the program is to sift out varieties which are high enough in essential food factors to make fluctuations of this nature of less significance and to insure optimum intake to the consumer.

SUMMARY

Four varieties of tomatoes were grown in 1938 and 1939 and four varieties of cabbages were grown in 1939 in four widely separated localities in the State of Maine to determine the effect of geographical situation upon the production of vitamin C.

The tomato varieties chosen were Penn State Earliana, Bestal, Best of All, and Comet. In preliminary tests the first two were relatively low in ascorbic acid; the other two were at the upper end of the scale.

In 1938 it was observed that the tomatoes grown in Aroostook were lower in ascorbic acid content than those produced in the three more southern localities. The majority of the samples from Orono, Highmoor, and Kennebunk were over 0.06 mg. per gram greater in vitamin C content than those from Aroostook.

In 1939 the observations were continued with the experimental procedure unchanged. The tomatoes grown in Aroostook were lower in ascorbic acid than those grown in Orono but higher than those grown at Highmoor and Kennebunk. The majority of the samples were more than 0.04 mg. per gram higher in ascorbic acid content in Aroostook, Orono, and Highmoor than in Kennebunk.

An analogous experiment in which four varieties of cabbages were used for the test crop confirmed the 1939 evidence obtained from tomatoes.

The above results demonstrated that environmental agencies markedly influence the synthesis of vitamin C in tomatoes and cabbages, and that geographical situation is not a contributing factor except insofar as environmental conditions are consistently characteristic of that situation.

An analysis of available weather data provided a certain basis for the assumption that sunlight, rainfall, and probably temperature may all be causal agents in the variations in ascorbic acid.

Paralleling maturation of the tissue, a definite rise in ascorbic acid concentration in the tomato and a decline in the cabbage was observed. These phenomena were related to geographical situation to the extent that maturity rate was hastened or delayed by the climatic conditions prevailing throughout the growing season in any one region.

Although variations effected by environment may be of greater magnitude than varietal differences, this does not lessen the importance of the varietal values. It is obvious that if a variety of low ascorbic acid content is subjected to adverse environmental influences, the tomato may well be rendered useless as a source of vitamin C. On the other hand, a high vitamin variety subjected to the same unfavorable conditions could still contribute materially to vitamin C requirements.

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HEIGHT OF CORN AS A FACTOR IN EGG LAYING BY THE EUROPEAN CORN BORER MOTH IN THE ONE-GENERATION AREA[1]

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INTRODUCTION

Every investigator who has had occasion to record the number of egg masses of the European corn borer (*Pyrausta nubilalis* (Hbn.)) laid on plots of corn (*Zea mays* L.) of varying height within experimental fields has noted that where one generation occurs the tall corn receives more eggs than the short corn. The economic significance of this fact lies in its relation to the recommendation that corn be planted late as a control measure against the corn borer. Corn in fields planted late obviously would have shorter stalks at the time the eggs are laid than corn in fields planted early, and might show the same lower attractiveness to the ovipositing moths that has been noted in the late-planted corn in planting-date plot experiments. Since the comparative number of eggs deposited is one factor to be considered in the formulation of a reliable recommendation respecting the best time to plant corn to escape borer damage, it was deemed worth while to study the comparative numbers of egg masses laid in fields of different corn heights over county-wide areas.

Available for this study were the data[3] obtained over the 4-year period 1930–33 from 1,600 fields of corn distributed in several States in the one-generation area of the European corn borer. The original field records on file at the Federal laboratory for European corn borer research at Toledo, Ohio, were used.

SURVEY METHODS

The four annual surveys made by the staff of the laboratory during this period to ascertain the abundance of the egg masses of the borer covered 36 counties or districts of Michigan, Ohio, and New York. Although the data obtained from one field afforded the primary sampling unit, two larger groups were used in the analyses. Ten fields constituted what is here called a unit area, and three unit areas covering the entire county or group of two counties comprised a

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[2] The author acknowledges the encouragement and helpful suggestions given by W. A. Baker and C. M. Packard during this study.

[3] These data were obtained under the direction of K. W. Babcock, entomologist, with the statistical assistance of M. T. Meyers, agent, during the annual surveys conducted by the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, to determine the intensity of egg deposition of the European corn borer.

county area. The data included in this analysis came from 159 unit areas within 53 county areas and 1 additional unit area. Six of these unit areas were surveyed in 1933 and the others in 1930, 1931, and 1932.

The same general procedure was followed each year. The survey for each county area was made by one man. He was given a map upon which 30 points were indicated by dots. In the placing of these dots each unit area had been divided into 10 nearly equal parts and a point selected along a road as near as possible to the center of each part. When approaching the point indicated on the map, the man selected the first cornfield encountered. Every third day over a period of 18 to 21 days he made counts of the egg masses on five widely separated plants from each of six sections of the field. By counting only fresh, white egg masses he was reasonably certain that they had been laid since the preceding count, as older masses are of a darker color owing to the development of the embryos within the eggs.

The average height of the corn was recorded each time a field was visited, by measuring the height of 10 single plants taken at random if the corn was drilled, or of the plants in 10 hills if the corn was planted in hills. In either case the measurement was from the ground to the tip of the longest portion of growth when the leaves were gathered in the arms and held upward.

ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG THE COUNTY AREAS

In the first analysis of the data the 53 county areas were divided into 3 groups of 13 and 1 group of 14 on the basis of the average height of corn in the area. The areas having the shortest, below-average, above-average, and tallest corn plants, respectively, provided the data for these 4 groups. Within these groups another grouping of the fields was made, depending again on the height of the corn. As each county area included 30 fields, a group was made for each area of the 10 fields with the shortest corn plants. Lastly, the data were arbitrarily divided into those obtained during the first half and those obtained during the last half of the oviposition period. For these final subgroups the mean corn heights and the mean numbers of egg masses per 100 plants were calculated. The results are given in table 1.

It may be noted in table 1 that about twice as many egg masses were found in the county areas with corn above average in height as in the areas with below-average corn. If the higher levels of egg masses were the result of greater numbers of moths, were the moths attracted to the areas of thriftier, taller corn from areas of shorter corn, or did they survive from higher levels of fall populations of borers that tended to accumulate in areas of tall corn? Regarding the latter possibility data show that higher levels of egg masses in areas of tall corn were not preceded by higher levels of fall populations of borers. Supplementary data from the 27 county areas of above-average corn show that the populations of borers in the fall preceding the infestations of egg masses averaged 29.1 per 100 plants as compared with 42.6 from the 26 counties of below-average corn.

For a study of the factors associated with higher levels of fall populations of borers in areas of shorter corn, the data from certain counties grouped according to locality were available. The data taken in 1931

TABLE 1.—*Number of egg masses of the European corn borer on corn in the fields with shortest corn grouped according to county areas in Michigan, Ohio, and New York, 1930–33*

Corn-height group	County areas in group	Part of oviposition period	Mean date of record	Total egg masses per 300 plants	Mean corn height in county areas	Ten fields in county area with shortest corn		
						Mean egg masses per 100 plants		Mean corn height
	Number			*Number*	*Inches*	*Number*	*Percent of total*	*Inches*
Shortest	13	First half	July 7	41.1	30.33	4.8	11.7	23.0
		Last half	July 16	34.7	43.07	6.1	17.6	34.4
		Mean or total		75.8	36.70	10.9	14.4	28.7
Below average	13	First half	July 7	42.6	34.87	6.4	15.0	26.9
		Last half	July 16	31.8	50.57	6.0	18.9	40.4
		Mean or total		74.4	42.72	12.4	16.7	33.6
Above average	13	First half	July 5	118.4	41.83	25.1	21.2	34.1
		Last half	July 15	55.7	60.17	15.1	27.1	50.2
		Mean or total		174.1	51.00	40.2	23.1	42.2
Tallest	14	First half	July 4	102.4	49.00	25.4	24.8	41.9
		Last half	July 13	44.0	70.93	13.7	31.1	62.3
		Mean or total		146.4	59.97	39.1	26.7	52.1

and 1932 were used, because 18 of the same counties were surveyed both years, whereas only 8 of the same counties were surveyed in all 3 years. The counties surveyed may be considered in three groups on the basis of height of corn. The tallest corn was produced in a block of counties in northwestern Ohio. Corn intermediate in height was grown in counties bordering Lake Erie or separated from it by not more than 1 county in northwestern Ohio or southeastern Michigan and located north and east of the counties in the first group. The shortest corn was grown in New York State in counties bordering Lake Ontario or Lake Erie. The counties in each group are given in table 2 together with the mean corn heights, the mean numbers of egg masses per 100 plants, and the fall populations of borers. To assist in interpretation of the data the numbers of eggs per mass, the numbers of

TABLE 2.—*Mean heights of field corn, mean numbers of egg masses per 100 plants, and related data, in grouped counties differing in corn height, Michigan, Ohio, and New York, 1930–32*

Counties as grouped	Year	Mean height of corn	Egg masses per 100 plants	Eggs per mass	Eggs per 100 plants	Borers surviving	Borers per 100 plants
		Inches	*Number*	*Number*	*Number*	*Percent*	*Number*
Tall corn—Williams, Defiance, Paulding, Van Wert, Fulton, Henry, Wood; all in Ohio	1930						9.0
	1931	66.4	33.2	12.2	405	6.5	26.3
	1932	60.5	50.3	22.5	1,132	3.2	35.8
	Average	63.5	41.8	17.4		4.9	[1] 31.1
Intermediate corn—Lenawee and Monroe, Mich.; Lucas, Ottawa, Sandusky, Erie, and Huron, Ohio	1930						20.7
	1931	55.7	33.3	14.5	484	9.4	45.3
	1932	51.9	152.1	23.1	3,518	1.4	49.0
	Average	53.8	92.7	18.8		5.4	[1] 47.2
Short corn—Chautauqua, Jefferson, Wayne, and Monroe; all in New York	1930						24.0
	1931	39.5	31.6	15.1	477	23.3	111.5
	1932	42.7	21.7	27.5	598	13.8	82.6
	Average	41.1	26.7	21.3		18.6	[1] 97.1

[1] Average for 1931 and 1932 only.

eggs per 100 plants, and the percentages of borers surviving from the numbers of eggs laid are included.

Averages of 22 and 15 fields per county were surveyed in June and July of 1931 and 1932, respectively, to determine the number of egg masses per plant sample. The average number of eggs per mass was determined on the basis of samples of 100 masses taken, in lots of 25, 4 times during the season from each country or area where 30 fields were surveyed. In the fall 20 or 25 fields in each county were surveyed to determine the fall populations of borers, but no attempt was made to use the same fields that were used for the earlier counts.

In 1931 the shortest corn averaged 26.9 and 16.2 inches shorter than the tallest and intermediate corn, respectively, but the population of borers in corresponding counties the preceding fall was 166.7 and 15.9 percent more dense. In 1932 the shortest corn averaged 17.8 and 9.2 inches shorter than the tallest and intermediate corn, respectively, and the population of borers the preceding fall was 324.0 and 146.1 percent more dense. Although data are not available to compare the heights of corn in 1933 with the fall populations of borers in 1932, nevertheless the fall population of borers in the shortest corn in 1932 was 130.7 and 68.6 percent more dense than in the tallest and intermediate corn, respectively. The higher levels of fall population of borers in the shortest corn in 1931 and 1932, as may be noted from table 2, were the result of more eggs per mass and a much higher borer survival in the areas of shortest corn.

It is conceivable that the number of egg masses laid in an area might be determined by the size of the mass. If conditions caused the laying of smaller masses in one area than in another, more masses might be laid in that area provided the complement of eggs of the moths was not less. The difference between 27.5 and 22.5, the numbers of eggs per mass in 1932 in the areas of shortest and tallest corn (table 2), is not great enough to account for the larger number of egg masses in the areas of tallest corn. It appears possible that in the areas of shortest corn less favorable conditions prevailed during the pupation period, as well as during the dormant period of the borers, and reduced the fecundity of the females. Weather during moth flight is also believed to determine the percentage of the complement of eggs that are laid.

Houser and Huber [4] state:

> Since moths are attracted more strongly to the best corn it would appear logical to assume that if the fields of good corn were widely separated the moths would necessarily fly greater distances to satisfy their reproductive urge, for the fulfillment of this urge is imperative for the success of the species.

While the author does not hold the view that moths will fly great distances to seek good corn, it appears that fields within and near the border of an area of tall corn would undoubtedly attract some moths from an adjacent area of less thrifty corn. How far moths from outside would be attracted to fields within an area is not known. One or more of the several factors considered in the foregoing discussion might have contributed toward a higher level of egg masses in northwestern Ohio and southeastern Michigan, where the corn was relatively tall during moth flight, than in the counties bordering Lake Ontario in New York, where the corn was short.

[4] HOUSER, J. S., and HUBER, L. L. NUTRITION AS A FACTOR IN THE RESPONSES OF THE EUROPEAN CORN BORER. Jour. Econ. Ent. 22: 171–174. 1929.

ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG FIELDS WITHIN THE UNIT AREAS

PRELIMINARY ANALYSES

The preceding data show that factors other than height of corn probably caused greater numbers of egg masses to be laid in groups of counties with tall corn as compared with groups of counties with shorter corn. The relationship between the number of egg masses and the height of corn was studied also on the basis of field data taken in most cases from areas covering only a third of a county. Before this study was made five preliminary analyses were necessary.

(1) The first of these analyses eliminated from further consideration the data from 40 of the 160 unit areas available. The 40 unit areas having less than 5.1 egg masses per 30 plants were not used, because many of the fields were not infested. In unit areas where the infestation averaged less than 1 egg mass per 30 plants, only 32.5 percent of the fields were found to be infested; where the density of infestation averaged from 1 to 1.9 masses per 30 plants, 60 percent of the fields were infested; and where the infestation averaged from 4 to 5.9 masses per 30 plants, 93.5 percent of the fields were infested.

(2) It was determined that the data were homogeneous with respect to the distribution of the heights of the corn plants among the fields within the areas. This was accomplished by obtaining the difference between the height of corn in the tallest and shortest fields within each unit area. The absence of a significant change in this difference was indicated when the regression of the differences on the mean heights of corn in the unit areas was calculated. The coefficient of regression (b) was found to be $+0.005 \pm 0.0656$ inch. When the data were classified according to years, it was found that for 1930, 1931, and 1932 the difference in heights averaged 24.0, 21.5, and 26.0 inches, respectively. Since these averages differ only slightly from the 24-inch average for the data of all unit areas, and since the regression coefficient did not differ significantly from zero, it appears that during these years there was no marked tendency for a narrowing of the range between the heights of the corn in the field of tallest corn and in the field of shortest corn such as might be expected in years when the weather necessitated the planting of corn within a short period late in the season. In this respect, then, the data from the 120 unit areas may be regarded as one homogeneous set.

(3) It was determined that the regression of the number of egg masses per 30 corn plants on the height of corn for fields within the unit areas was on an average proportional to the mean number of masses found in the 10 fields examined in each area. The proportional relationship would necessarily occur in the absence of disturbing factors because of the mathematical considerations. An average increase of 1 egg mass per 30 plants from field to field for each increase of an inch in the height of the corn becomes an increase of 2 masses under conditions of infestation with twice the former number of egg masses in each field. This relation is purely mathematical. Increasing proportionally the magnitude of each item of the dependent variable while each item of the independent variable remains the same causes a proportional increase in the regression coefficient. This phase of the analysis was continued to determine if disturbing factors were involved,

Accordingly, the regression (b) of the number of egg masses per 30 plants in the individual fields (Y) on the height of corn in the fields (X) was calculated for each unit area. If it is assumed that the regression coefficients obtained were in fact proportional to the mean number of egg masses in each area, and each regression coefficient is then expressed as a percentage of the mean number of masses, the effect of egg-mass density may be disassociated from the effect of other possible factors on the regression coefficients. As subsequent data show, the mean level of corn height in the unit areas was the only other factor associated with the comparative regression coefficients. The comparative regression coefficients were therefore corrected for the effect of their regression on the mean level of corn height and converted back to b values not expressed as percentages of the mean number of masses.

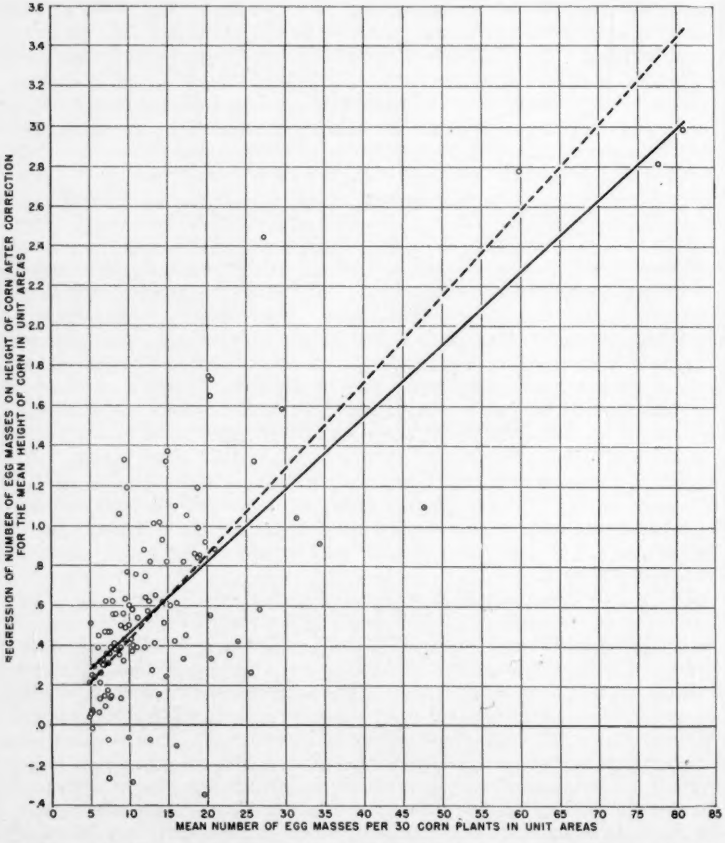

FIGURE 1.—Coefficient of regression of Y on X within unit areas plotted against the mean level of egg masses after correction of the coefficient for the mean height of corn. The unbroken line is the regression line as calculated; the broken line is the regression line expected on the basis of proportionality between the regression coefficients and the mean level of egg masses.

In figure 1 the corrected b values are plotted against the mean numbers of egg masses in the unit areas. The unbroken regression line was fitted to the plotted points by the method of least squares.

The next step was to compare the regression of the corrected b value on the mean level of egg masses with the regression expected on the basis of a proportional relationship between the two factors. The corrected b values were found to increase on an average 0.0362 ± 0.00296 for each increase of 1 egg mass per 30 plants. The mean number of masses per 30 plants in the 120 unit areas was 14.23. The mean of the corrected b values was calculated to be $+0.612 \pm 0.0350$. Exact proportionality would yield a straight line passing through the origin and the general mean. Its slope would then be 0.0430 ± 0.00246. The broken line in figure 1 shows this expected increase. The difference between this expected increase and the actual increase is 0.0068 ± 0.00385, giving a t value of 1.766, which is somewhat less than 1.980, the value required for significance. It appears that the corrected b values show a closely proportional relationship to the mean level of egg masses in the unit areas.

An inspection of figure 1 suggests that the meagerness of the data between levels of 35 and 82 egg masses might have had an undue effect on the position of the regression lines. Accordingly, the data were recalculated with the exclusion of the data from the 4 unit areas between those levels. The corrected b values increased on an average 0.0387 ± 0.00559 for each increase of 1 egg mass per 30 plants as compared with an expected increase of 0.0442 ± 0.00284 on the basis of proportionality. The difference of 0.0055 ± 0.00627 is not significant. The exclusion of the 4 unit areas strengthens the previous conclusion.

(4) It was determined that no other factors were involved in the association of the dependent with the independent factor shown in figure 1. The effect of the mean level of corn height has been accounted for in the construction of figure 1. Other possible factors are location of the unit areas and the year in which the data were obtained. The variability among the 120 corrected regression coefficients was compared with the error of sampling within the unit areas, after the variability among the coefficients due to the proportional relationship with the mean level of egg masses had been taken out. The standard error of the coefficients around the unbroken regression line in figure 1 is 0.3831. The variance of the regression of Y on X was calculated for each unit area, and from the mean variance the generalized standard error of b was found to be 0.4117. The near equality of these two values led to the conclusion that, within the several States of the one-generation area, including the unit areas, and within the period covered, factors other than the general level of corn height for which the regression coefficients were corrected had no effect on the regression of Y on X.

(5) A study was made to determine whether the relation between number of egg masses per 30 corn plants and the height of corn among fields within the unit areas was linear or curvilinear. Because of the extreme variability of the data, a grouping of the unit areas was necessary to stabilize the data sufficiently to make the study. The corn-height data were arranged according to the average height of corn for each of the 120 unit areas. Different height levels were then obtained by dividing the unit areas into 20 equal groups. Then the fields in each unit area were grouped according to their deviation

from the mean height. Four classes were made with intervals of 4.0 inches, and 2 other classes included any fields deviating either positively or negatively by more than 8.0 inches from the mean height. The number of egg masses per 30 plants in each field was expressed as a percentage of the mean number in the 10 fields of each area. Then the corn-height deviations of the fields included within each of the 6 height classes for each of the major groupings of the unit areas were averaged. The corresponding number of egg masses on a percentage basis were also averaged. A visual examination of the plotted data indicated a linear regression of number of egg masses on corn height. It appears, therefore, that because the relation between the two variables was linear within areas as few as 6 unit areas, it is reasonably safe to conclude that such would have been the case within individual unit areas if the sampling had been more intensive within the areas.

REGRESSION OF NUMBER OF EGG MASSES ON HEIGHT OF CORN

The information gained from the preceding analyses assures that the study of the regression of the number of egg masses per 30 plants on the height of corn from field to field within the unit areas may proceed on a linear basis with all other factors accounted for, when the regression coefficients of Y on X are converted to percentages of the mean numbers of egg masses in the unit areas. An average difference of 24 inches between the tallest and shortest corn within the unit areas was found regardless of the mean level of corn height.

The comparative regression coefficients are plotted in figure 2

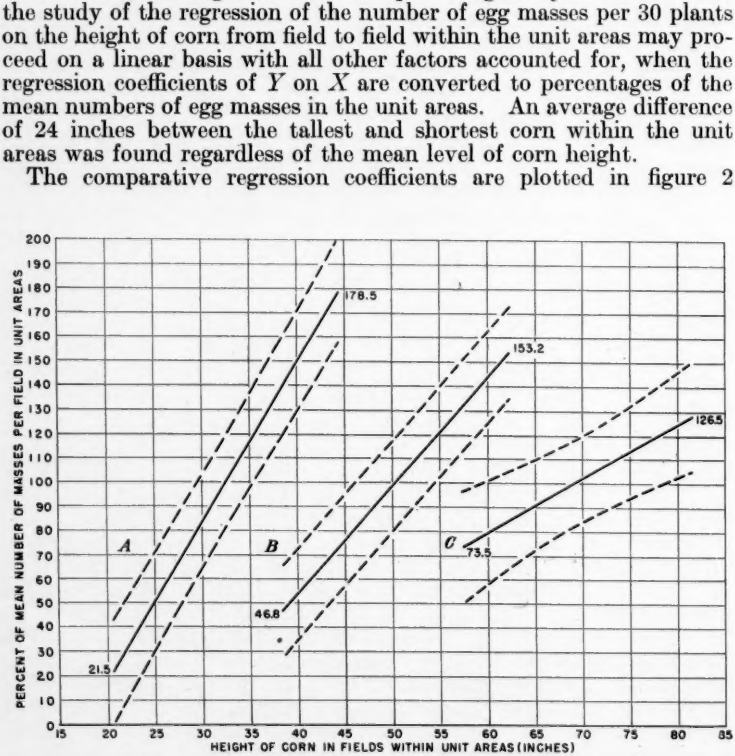

FIGURE 2.—Coefficient of regression of Y on X within unit areas, expressed as a percentage of the mean of Y, plotted against the mean height of corn. The broken-line curves give the limits (for odds of 19 to 1) enclosing the true regression line whose estimate is the unbroken line.

against the mean height of the corn in the fields within the unit areas. For each increase of an inch in the general level of corn height the coefficient of regression (b) of Y on X, expressed as a percentage of the mean of Y, was found to decrease 0.117 ± 0.0277. With 118 degrees of freedom the regression may be regarded as highly significant. The standard error of the plotted points around the regression line was found to be 2.931 percent as compared with a within-unit-area error of sampling for b of 2.810 percent. The mean of the dependent variable is 4.43 ± 0.072 percent, and that of the independent variable is 50.4 inches.

The next step is to use the information given by the data in connection with figure 2 to show the average conditions encountered within unit areas having specified levels of corn height. Mean heights of 32.4, 50.4, and 69.4 inches were selected. At these levels the best estimates of the regression of Y on X are calculated to be 6.54 ± 0.566, 4.43 ± 0.268, and 2.21 ± 0.526 percent, respectively. Within unit areas where the corn averaged about 32.4 inches in height, an average of $100-12(6.54)$, or 21.5 percent of the mean number of egg masses, was counted in the fields with shortest corn as compared with $100+12(6.54)$, or 178.5 percent of the mean number of egg masses, in the fields with tallest corn. In these areas on an average 12.0 percent as many masses were counted on the shortest corn as on the tallest corn. Similarly, within unit areas averaging about 50.4 inches in corn height an average of 46.8 percent of the mean number of egg masses was counted in the fields with shortest corn as compared with 153.2 percent in the fields with tallest corn, or 30.5 percent as many in the fields with shortest corn. Within unit areas averaging about 69.4 inches in height an average of 73.5 percent of the mean number of egg masses was counted in the fields with shortest corn as compared with 126.5 percent in the fields with tallest corn, or 58.1 percent as many in the fields with shortest corn.

The foregoing percentages of the mean number of egg masses are plotted in figure 3 against the height of the corn in the fields of shortest and tallest corn, respectively, within a unit area and are connected by straight unbroken lines. The vertical scale represents numbers of egg masses as percentages of the mean number of masses in all fields within a unit area. Hence the mean number of masses per field is 100 percent on the scale. The midpoint of each regression line coincides with the point determined by plotting 100 percent against the mean height of corn. The length of the lines covers a range extending from 12 inches below to 12 inches above the mean height. The range of 24 inches in height was the average difference between the field of shortest corn and the field of tallest corn within a unit area.

The general level of corn height within a unit area has been shown to have a highly significant effect on the uniformity of the distribution of the egg masses among the fields. When the corn height averaged 32.4 inches, only 12.0 percent as many masses were counted on the shortest corn as on the tallest corn, as compared with 58.1 percent as many when the corn averaged 69.4 inches high. This difference may be attributed to some factor associated with the difference of 37 inches in the general level of corn height. This factor might be explained as a decreasing difference in the attractiveness to the corn borer moth between fields of tall corn and fields of short corn as the general level of the height of corn in the fields within an area increased.

This explanation is in harmony with the increased attractiveness of the fields of shortest corn during the last half of the oviposition period. The data given in table 1 show that 23.7 percent of the masses laid during the last half of the oviposition period were laid in the third of the fields with the shortest corn as compared with 18.2

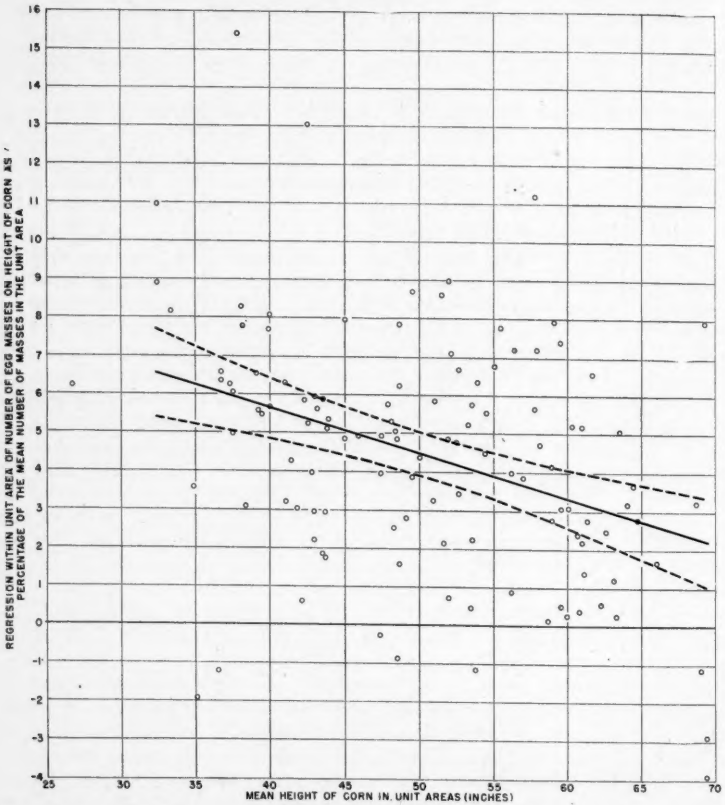

FIGURE 3.—Percentages of the mean number of egg masses of the European corn borer per cornfield in unit areas that were laid in single fields of different corn height, when the mean height of the corn in all fields in the unit areas approximated (*A*) 32.4, (*B*) 50.4, and (*C*) 69.4 inches. The broken curves give the limits enclosing the true regression line (for odds of 19 to 1) when the egg masses on 30 plants in each of 36 fields taken at random within the unit areas are counted and the level of egg density ranges from 10 to 22 egg masses per 30 plants.

percent laid in the same fields during the first half of the period. That is, there was a partial shift in egg laying away from the fields of taller corn toward the fields of shorter corn as the season advanced and the general level of corn height increased. On the basis of the total number of egg masses laid during the oviposition period, and in harmony with this phenomenon, the fields of shorter corn appear to

have attracted proportionately more moths when the general level of corn height was high than when the general level was low. Accordingly, the distribution of the egg masses among the fields was more uniform within areas of tall corn.

The attractiveness to the corn borer moth of corn in late-planted fields as compared with corn in fields planted earlier is one of the four major factors [5] to be considered in recommendations for the best time to plant corn to escape maximum damage by the borer. Although the planting dates for the fields surveyed in the present study were not determined, it is obvious that the earliest planted fields within an area would be in the group with tallest corn at the time of moth flight, whereas the latest planted fields would be among the fields in the group with shortest corn. The fields of shorter corn received, in general, smaller numbers of egg masses within 112 of the 120 unit areas studied. Hence late-planted fields had some advantage over early-planted fields from this factor alone. If the conditions giving the results obtained from 1930 to 1933 may be considered as typical, the advantage of late-planted fields would be greatest in the localities and years when the level of corn height is comparatively low at the time of moth flight. In areas where the corn was short at the time of moth flight, 8.3 times as many egg masses were laid in the fields of tallest corn as in the fields of shortest corn, whereas in areas where the corn was 37 inches taller at the time of moth flight only 1.7 times as many masses were laid in the fields of tallest corn as in the fields of shortest corn. The advantage of the latest over the earliest planted fields in this case was only 20.5 percent as great in the areas with a high level of corn height as in the areas with a low level of height.

ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG PLOTS WITHIN FIELDS

Consistent decreases in the number of eggs laid with decreases in the height of corn among plots occurred in experiments carried on by Neiswander and Huber.[6] In the present study plot experiments conducted by the Illinois Natural History Survey and the Illinois Agricultural Experiment Station from 1929 to 1934 were available for determining the association of number of egg masses with height of corn among plots within a field. Each plot was 9 hills in size during 5 seasons and 12 hills during 1 season. The number of plots in the field during the period ranged from 64 to 132. The height of the corn in the plots, measured from the ground to the tip of the longest portion of growth of the hill when the leaves were held upward, varied because of the strain of corn, the date of planting, and the location of the plot in the field. The number of egg masses laid in each plot was found by examining all plants at least once every 5 days and identifying them with clips so that the same masses would not be counted twice.

The data were considered as one set of data irrespective of strain or other factors. The mean number of egg masses (Y) was found to bear a linear relationship to the height of corn (X). The coefficients of regression of Y on X were calculated and adjusted to express the change in the number of egg masses per 30 plants for each change of

[5] The other factors are being considered in supplementary studies.

[6] NEISWANDER, C. R., and HUBER, L. L. HEIGHT AND SILKING AS FACTORS INFLUENCING EUROPEAN CORN BORER POPULATION. Ent. Soc. Amer. Ann. 22: 527–542, illus. 1929.

1 inch in the height of corn. The adjusted values were then expressed as percentages of the respective mean number of egg masses per 30 plants in all plots. A comparison between the plot data within fields and the field-to-field data, taken from figure 2 for equivalent heights of corn, is given in table 3.

TABLE 3.—*Regression of number of egg masses of the European corn borer per 30 plants of corn, expressed as a percentage of the mean number of masses, on the height of corn among plots within fields, compared with the regression among fields, near Toledo, Ohio, 1929–34*

Year	Mean date of oviposition	Among plots within fields			Among fields (readings taken from the unbroken line in fig. 2)	
		Estimated mean height of corn ($\bar{X}$) on mean date of oviposition	Mean number of egg masses per 30 plants ($\bar{Y}$)	Regression of Y on X	Mean height of corn ($\bar{X}$) during oviposition period	Regression of Y on X
		Inches	*Number*	*Percent*	*Inches*	*Percent*
1929	July 8	38.8	11.6	6.44±0.506	38.8	5.79
1930	July 4	40.0	24.2	5.05±0.449	40.0	5.65
1932	July 2	52.2	14.1	3.07±0.203	52.2	4.22
1931	July 5	59.5	4.7	4.31±0.576	59.5	3.37
1933	July 4	59.6	16.4	2.61±0.597	59.6	3.35
1934	July 2	62.5	8.6	2.88±0.283	62.5	3.01
Mean	July 4	52.1	13.3	4.06±0.188	52.1	4.23

As shown in table 3, for each increase of 1 inch in corn height from plot to plot within fields there was an average increase of 4.06±0.188 percent, or 0.54 egg mass, in the mean number of egg masses per 30 plants. An average increase of 4.23 percent is shown between fields having average equivalent heights of corn. Hence it is concluded that moths of the corn borer are attracted as much more to fields of tall corn than to fields of short corn as they are to plots of tall corn than to plots of short corn within fields.

SUMMARY

In the one-generation area of the European corn borer (*Pyrausta nubilalis* (Hbn.)) investigators have found that plots of tall corn always contain more egg masses than plots of short corn. Because of the relation of height of corn to time of planting, this fact is important when control measures are being considered. A study has therefore been made of the distribution of egg masses among fields of corn over county-wide areas.

The egg masses were counted on 30 corn plants in each of 10 cornfields taken at random in each of 160 unit areas in Michigan, Ohio, and New York from 1930 to 1933, inclusive. A unit area was usually a third of a county or a group of two counties. The average plant height during the oviposition period was determined for each field.

Data from unit areas within which all the fields examined were infested showed that factors such as location of the unit areas and year in which the data were obtained did not affect the distribution of egg masses among the fields within the unit areas. The mean level of corn height, however, was found to have a marked effect on the distribution of egg masses among the fields.

More egg masses were laid in the fields of tall corn within an area than in the fields of short corn, but the difference was less marked in

areas where the general level of corn height was high. When the corn within unit areas averaged 32.4 inches in height, 8.3 times as many egg masses were laid in the fields of tallest corn as in the fields of shortest corn, whereas in unit areas where the corn averaged 69.4 inches at the time of moth flight only 1.7 times as many egg masses were counted in the fields of tallest corn.

When the data were grouped on the basis of counties without regard to the unit areas within them, about twice as many egg masses were found in the counties with corn above average in height as in the counties with below-average corn. This condition did not seem to result from higher levels of early summer infestations of egg masses in areas of tall corn preceded by higher levels of fall populations of borers. Other factors are discussed.

Moths were found to be attracted as much more to fields of tall corn than to fields of short corn as they were to plots of tall corn than to plots of short corn within experimental fields.

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COMPARATIVE RIPENING OF BEEF FROM GRASS-FATTENED AND GRAIN-FATTENED STEERS [1]

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INTRODUCTION

Cattle fed and finished on grass usually bring a lower price on United States markets than similar cattle finished on grain. The reasons advanced for the price discrimination are many. One of the principal objections raised is that the beef from cattle fattened on grass does not keep or ripen satisfactorily. In order to obtain much-needed information on the "grass beef" problem, a cooperative study was made by the Virginia Agricultural Experiment Station, the Virginia State Division of Markets, and the United States Department of Agriculture Bureau of Animal Industry and Agricultural Marketing Service. The first experiment was conducted in 1937–38 and was followed by similar experiments in 1938–39 and 1939–40. The present paper reports the phase of this study concerned with the comparative ripening qualities of the two kinds of beef.

METHODS OF EXPERIMENTATION

Beef-type steers produced in southwestern Virginia and averaging low Good in grade of feeder were selected for this study. In each of the 3 experiments, 40 steers about 2½ years old and of similar breeding, weight, and condition were used. All cattle were wintered in 1 group on a medium plane of nutrition, and they lost an average weight of approximately 100 pounds.

At the beginning of the grazing season the cattle were divided into 2 comparable groups of 20 head each. Group 1 was turned on bluegrass (*Poa pratensis*) pasture and received only grass and block salt for approximately 5 months. Group 2 was placed in dry lot and fed shelled corn, cottonseed meal, and mixed hay. These cattle had access to block salt and were fed to the same degree of fatness as those on grass. The feed required consisted of 649 pounds of corn, 80 pounds of cottonseed meal, and 412 pounds of hay per 100 pounds of grain. At the end of the feeding period each group was divided as equally as possible into two comparable subgroups of 10 animals each. One subgroup in each instance was shipped to the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., and the other to the Jersey City market.

[1] Received for publication October 23, 1941.

At Beltsville the cattle were slaughtered and the carcasses were chilled to approximately 34° F. Ether extract of the eye muscle of the ninth-tenth-eleventh-rib cut was determined for each of the 20 carcasses. Three pairs of these carcasses were selected for the study reported in this paper. Each pair consisted of a carcass of a grass-fed steer and that of a grain-fed steer. The pair mates were of as nearly the same fat content of eye muscle as was possible to obtain from the 10 carcasses representing each type of feeding. From these 3 pairs of carcasses the 12 sixth-seventh-eighth-rib cuts were used for the study on ripening.

Beginning 15.7 days after slaughter, on the average, in the 3 experiments, the analytical work on the rib samples from the right side of the 6 carcasses was begun. The eye muscle was removed from the sixth-rib cut, ground 3 times, and mixed thoroughly for chemical analysis. Preparatory to determination of the expressible-juice content and to tests for flavor and aroma, the seventh-eighth-rib cut was cooked at an oven temperature of 125° C. to an internal meat temperature of 58° C. The juice content was determined by a method, developed by the Bureau of Animal Industry, which involves the testing of small samples of cooked meat in a hydraulic press under certain conditions of temperature, pressure, and time.[2] Flavor and aroma were judged by a committee of qualified persons.

To subject the meat to an extremely critical test, the sixth-seventh-eighth-rib cuts from the left side of the carcass were stored at 33° to 36° F. for 50 days. At the end of this period, all moldy and slimy surfaces were removed from the samples. The same procedure was then followed as described for the 15.7-day period.

To measure the autolysis, or break-down, of muscle tissue during ripening, the content of amino and nonprotein nitrogen, as well as that of other tissue components, was determined. Moisture, ether extract, total nitrogen, amino nitrogen (by formol titration), and ash determinations were made according to methods recommended by the Association of Official Agricultural Chemists.[3] Nonprotein nitrogen was determined by treating 5 gm. of ground meat with 100 cc. of 10-percent trichloroacetic acid. Sulfydryl determinations were made according to the Okuda iodate method [4] with the use of Lavine's modification.[5] Reducing substances were determined by the Somogyi-Shaffer-Hartmann method, as given in Hawk and Bergeim.[6] Although reducing values were obtained by this method, the values are expressed as total reducing substances in terms of glucose. The reduction obtained is probably due to intermediate products formed during the conversion of glycogen. The data obtained on total nitrogen, amino nitrogen, nonprotein nitrogen, and sulfydryl were analyzed statistically by the use of Fisher's *t* test.[7]

[2] The method used for determining expressible juice was that briefly given in the Reports of the Chief of the Bureau of Animal Industry for 1937 and 1939, and later improved. A paper giving the details of the method has been prepared for publication.

[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus. Washington, D. C. 1935.

[4] OKUDA, YUZURU. A NEW METHOD FOR THE DETERMINATION OF CYSTINE IN PROTEINS (THE IODINE METHOD). Jour. Biochem. 5: 217–227. 1925.

[5] Personal communication from T. F. Lavine, Lankenau Hospital Research Institute, Philadelphia, Pa.

[6] HAWK, PHILIP B., and BERGEIM, OLAF. PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ed. 11, 968 pp., illus. Philadelphia. 1937.

[7] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

EXPERIMENTAL RESULTS

The paired samples of the ninth-tenth-eleventh-rib cuts used in the 3-year study were of approximately equal fatness, as indicated by the ether-extract content of the eye muscle. The differences in the ether extract among the nine pairs at time of slaughter ranged from 0 to 0.09 percent, the mean difference being 0.051 percent. Table 1 presents data on the comparative composition of the beef after ripening for an average period of 15.7 days. These data show that the beef from the grain-fed and grass-fed steers differed little in moisture, ash, protein, and ether extract. Any changes in these constituents that occurred during the short period of ripening were similar for the two types of beef. In the amino-nitrogen determinations, there was no difference, on the average, between the meats representing the two types of fattening. For each of the 3 years, as well as for the average of the 3 years, there were only small differences in nonprotein nitrogen. The total reducing substances show no appreciable difference, but the percentage of sulfydryl in the beef from the grass-fed cattle was appreciably higher than that in the grain-fed cattle. However, the higher sulfydryl content was not interpreted as indicating that more ripening had occurred in the beef from the grass-fed cattle.

Table 1 also presents data on the 50-day ripening period for the same constituents. As was found after the short period of ripening, the differences between the two kinds of beef in moisture, ash, protein, and ether extract were of minor importance. Furthermore, such small changes as did occur in these constituents as a result of the longer ripening period were similar in the beef from the grain-fattened and grass-fattened cattle.

On the other hand, the values for nonprotein nitrogen and amino nitrogen were considerably higher after 50 days of ripening than after an average of 15.7 days. The increase represents what normally occurs in ripening meat as a result of attack by enzymes and bears out the results of previous work. However, the important point is that the increase was similar in the two kinds of beef.

There was little difference, between the two kinds of beef, in total reducing substances and only a small increase as a result of a longer ripening period. Sulfydryl values increased between the 15.7- and 50-day intervals somewhat more rapidly in the beef from grain-fattened cattle, with the result that there was less difference between the two kinds of beef after 50 days than after 15.7 days. During ripening, labile sulfur from the protein molecule splits off and is liberated as H_2S and other volatile compounds. The sulfydryl is probably derived from cystine, glutathione, and other sulfur-bearing amino acids that are liberated during autolysis.

With respect to the content of amino and nonprotein nitrogen (the indices of protein break-down) as well as total nitrogen, the differences between the two kinds of beef were found to have no statistical significance either for the 15.7- or the 50-day ripening period. Sulfydryl values, on the other hand, were significantly higher for the beef from grass-fattened cattle after 15.7 days of ripening, owing probably to the higher sulfydryl content of the grass ration. After 50 days, however, the difference was not significant. The latter

TABLE 1.—*Comparative chemical composition and percentage of expressible juice of beef from grain-fattened and grass-fattened steers after a short (an average of 15.7 days) and a long (50 days) period of ripening*

SHORT PERIOD OF RIPENING

Item	Data for indicated year and type of fattening											
	1937–38			1938–39			1939–40			3-year average		
	Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference
Animals.....number	3	3		3	3		3	3		9	9	
Ripening period.....days	18	18		16	16		13	13		15.7	15.7	
Moisture.....percent	72.73	73.15	−0.42	75.62	73.39	+2.23	72.54	72.26	+0.28	73.63	72.93	+0.6967
Ash.....do	1.087	1.110	−.023	1.067	1.050	+.017	1.200	1.190	+.010	1.1177	1.1167	+.0010
Protein.....do	23.12	22.39	+.73	21.81	22.72	−.91	22.47	22.74	−.27	22.47	22.62	−.1500
Ether extract.....do	4.62	4.67	−.05	2.50	3.05	−.55	3.26	3.97	−.71	3.46	3.90	−.4367
Total nitrogen.....do	3.70	3.58	+.12	3.49	3.58	−.09	3.60	3.64	−.04	3.60	3.60	−.0033
Nonprotein nitrogen.....do	.388	.401	−.013	.419	.418	+.001	.408	.421	−.013	.405	.413	−.0083
Nonprotein nitrogen as related to total nitrogen.....percent	10.50	11.21	−.71	11.99	11.67	+.32	11.33	11.58	−.25	11.28	11.49	−.2133
Amino nitrogen.....do	.0689	.0702	−.0013	.0601	.0596	+.0005	.0558	.0550	+.0008	.0616	.0616	0
Amino nitrogen as related to total nitrogen percent	1.86	1.96	−.10	1.72	1.66	+.06	1.55	1.51	+.04	1.71	1.71	0
Total reducing substances.....do	.1567	.1687	−.0120	.0597	.0587	+.0010	.1547	.1523	+.0024	.1237	.1266	−.0029
Sulfydryl.....do	.0303	.0560	−.0257	.0300	.0433	−.0133	.0312	.0441	−.0129	.0305	.0478	−.0173
Protein, fat-free.....do	23.85	23.16	+.69	22.14	23.38	−1.24	23.39	23.64	−.25	23.13	23.39	−.2667
Ratio of moisture to protein	3.15:1	3.27:1	−.12:1	3.47:1	3.23:1	+.24:1	3.23:1	3.18:1	+.05:1	3.28:1	3.22:1	+.057:1
Expressible juice.....do	34.70	35.96	−1.26	40.20	39.70	+.50	44.80	48.50	−3.70	39.90	41.39	−1.49

LONG PERIOD OF RIPENING

Animals.....number	3	3		3	3		3	3		9	9	
Ripening period.....days	50	50		50	50		50	50		50	50	
Moisture.....percent	72.01	72.55	−0.54	72.59	72.31	+0.28	72.74	72.09	+0.65	72.45	72.32	+0.1300
Ash.....do	1.110	1.077	+.033	1.060	1.120	−.060	1.127	1.063	+.064	1.0989	1.0867	+.0122
Protein.....do	23.43	22.52	+.91	23.63	24.20	−.57	22.98	22.81	+.17	23.35	23.18	+.1700
Ether extract.....do	2.99	3.77	−.78	2.58	2.47	+.11	2.42	3.07	−.65	2.66	3.10	−.4400
Total nitrogen.....do	3.75	3.60	+.15	3.78	3.87	−.09	3.68	3.65	+.03	3.74	3.71	+.0300
Nonprotein nitrogen.....do	.525	.504	+.021	.505	.505	0	.533	.515	+.018	.521	.508	+.0130
Nonprotein nitrogen as related to total nitrogen.....percent	14.00	13.98	+.02	13.34	13.02	+.32	14.51	14.11	+.40	13.95	13.70	+.2467
Amino nitrogen.....do	.0966	.0981	−.0015	.1024	.0989	+.0035	.1019	.1029	−.0010	.1003	.1000	+.0003
Amino nitrogen as related to total nitrogen percent	2.58	2.73	−.15	2.71	2.55	+.16	2.77	2.82	−.05	2.68	2.70	−.0200
Total reducing substances.....do	.3640	.3690	−.0050	.0363	.0413	−.0050	.0790	.0697	+.0093	.1598	.1600	−.0002
Sulfydryl.....do	.1050	.1087	−.0037	.0664	.0728	−.0064	.0778	.0938	−.016	.0831	.0918	−.0087
Protein, fat-free.....do	24.27	23.42	+.85	24.30	24.79	−.49	23.73	23.77	−.04	24.10	23.99	+.1067
Ratio of moisture to protein	3.07:1	3.22:1	−.15:1	3.07:1	2.99:1	+.08:1	3.17:1	3.16:1	+.01:1	3.10:1	3.12:1	−.02:1
Expressible juice.....do	31.70	31.60	+.10	38.90	42.00	−3.10	47.00	46.60	+.40	39.20	40.06	−.86

finding indicates that ripening may produce volatile sulfydryl compounds and that some loss results. Except for sulfydryl, the small differences obtained in these constituents are believed to be within the range of experimental error.

After an average ripening period of 15.7 days, no difference was observed in the flavor and aroma of the cooked seventh-eighth-rib cuts from the two kinds of meat. After 50 days, the characteristic flavor and aroma of ripened beef were rather pronounced, but again no difference in flavor and aroma between the grain-fattened and grass-fattened beef was detected. The results after both short and long periods of ripening indicate that the expressible-juice content of the beef from grass-fattened cattle was not significantly different from that of the grain-fattened steers.

SUMMARY AND CONCLUSIONS

A 3-year study was carried on in 1937–40 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., to compare the ripening of meat from steers about 2½ years old fattened on bluegrass pasture with that from cattle fattened on corn, cottonseed meal, and hay when the two kinds of beef are of equal fatness. Rib cuts from 18 paired carcasses, representative of a total of 120 animals, were used during the 3 years. Pair mates of as nearly the same fat content of eye muscle as possible were selected. One sixth-seventh-eighth-rib cut from each carcass was ripened at 33°–36° F. for an average of 15.7 days; the other, for 50 days.

No significant difference was found in the rates of ripening of the beef from the two types of feeding. This result was shown chemically by data on basic composition and especially by determinations of amino and nonprotein nitrogen. The sulfydryl content of beef from grass-fattened cattle was the higher but had no bearing on the rate of ripening, inasmuch as the indexes of protein break-down were similar for beef from both types of feeding. The higher sulfydryl content of beef from grass-fattened cattle is probably due to the higher content of sulfydryl of the grass ration.

No difference was observed in the flavor and aroma or in the expressible-juice content of the two kinds of beef.

DESIGN OF EXPERIMENTAL COMPARISONS BETWEEN LINES OF BREEDING IN LIVESTOCK [1]

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INTRODUCTION

The comparison of animals representing different breeding is one of the most common forms of experimentation in the field of animal improvement. The characteristics for which they may be compared are many. Present emphasis on the development of selected inbred lines will result in a great increase in this type of experimentation if the possible uses of these new lines to the livestock industry are to be accurately evaluated. Large numbers of experimental comparisons between the various lines, the breeds in general, breed crosses, and crosses of lines both within and between breeds will be necessary if the most productive lines and crosses are to be identified. This type of work is and will remain subject to serious financial limitations because of the requirements for space, labor, and equipment. Therefore, it is highly important to make the most efficient possible use of experimental animals.

The purpose of this paper is to clarify the effects that varying numbers of males and females used to produce progenies will have on the efficiency with which experimental comparisons of such progenies can be made. A specific objective will be to answer questions of the following type: Will a comparison of two lines be made more efficiently by use of the offspring, in each line, of 2 sires bred to 10 females each or of 3 sires bred to 5 females each? The term "line" will be construed to include inbred lines, crosses between separate inbred lines or breeds, and the various breeds in general.

ELABORATION OF THE PROBLEM

The most efficient experiment for the comparison of two lines will be defined as the one that allows the smallest observed difference to be judged of sufficient size to indicate a real difference between the lines in question. The significance attached to any experimental difference is based on its size in relation to the random variation of differences of the same kind in repetitions of the experiment. Other things being equal, a difference between the means of 2 sets of 10 animals would always be considered more significant than a difference of the same size between the means of 2 sets of 5 animals because, being based on more animals, it would be less likely to have resulted from random variation.

Consider now the number of males and females to be used in producing samples of lines for comparison. It is clear that increasing the progeny of a single sire by breeding more females to him will increase

[1] Received for publication July 17, 1941. Paper No. 1901, Scientific Journal Series, Minnesota Agricultural Experiment Station. Data relating to swine were obtained in cooperation with the Regional Swine-Breeding Laboratory, Bureau of Animal Industry, U. S. Department of Agriculture.

the accuracy with which his breeding performance, when mated to females of a certain line, is measured. However, it is uncertain whether he is representative of his line and, therefore, whether his progeny adequately represents the line or cross being tested. Assurance that the sample is representative of the entire line will be attained only when the number of sires producing it is increased. Because the total number of experimental animals that can be raised is limited, it is important to know the balance between the number of progeny to be raised from each sire and the number of sires to use in each line which will give the most efficient experiment.

AN APPROXIMATE SOLUTION

DEFINITION OF DIFFERENCE NECESSARY FOR SIGNIFICANCE

A mathematical expression of efficiency (as herein defined) in terms of number of sires, dams, and progeny can be derived from the use of well-known statistical concepts.

The significance of a difference between the means of samples of animals from two different lines can be tested statistically by the use of "Student's" distribution, using the argument,

$$t=d/\sqrt{s_1^2+s_2^2}, \tag{1}$$

wherein

d=difference between the means of the two lines,

s_1=standard error of the mean of one line, and

s_2=standard error of the mean of the other line.

Then d, equal to $t\sqrt{s_1^2+s_2^2}$, defines the minimum difference necessary for significance if the value of t is inserted that has the maximum probability of occurrence by chance to be accepted as indicating a significant difference.

The variance of the mean of k full sibs can be expressed as

$$\sigma_1^2=K/k$$

where K is the variance of the full sibs. The variance of the mean of fk animals where k offspring are raised from each of f dams all bred to the same sire is

$$\sigma_2^2=\frac{F+K/k}{f}$$

where F is the variance of the paternal half sibs resulting from differences in dams or differences in environment to which full sibs are not subjected. Analogically the variance of the mean of mfk animals where k offspring are raised from each of mf dams, f of which are bred to each of m sires, is

$$\sigma^2=\frac{M+\frac{F+K/k}{f}}{m}$$

where M is the variance of the maternal half sibs resulting from sire differences.

Then

$$\sigma^2=\frac{M}{m}+\frac{F}{mf}+\frac{K}{mfk}$$

$$=M\left(\frac{1}{m}+\frac{1}{mf}\cdot\frac{F}{M}+\frac{1}{mfk}\cdot\frac{K}{M}\right)$$

In the above the symbols, σ, M, F, and K indicate parametric values.

Let the experiment comparing two lines be symmetrical; i. e., the values of m, f, and k shall be the same for both lines. Assume that M has the same value in each of the lines being compared and that this is likewise true for F. This is justifiable only in the absence of definite knowledge to the contrary. One might presume that equality of either of these variances for two lines would only rarely occur, but lacking knowledge based on previous experience, one would not know which of two lines was the more variable. The assumption of equality would then be more logical than any other specific relationship. Under this condition K will also be essentially equal for the two lines. Then substituting in equation (1), remembering that on the average $s^2=\sigma^2$,

$$d=t\sqrt{2M\left(\frac{1}{m}+\frac{1}{mf}\cdot\frac{F}{M}+\frac{1}{mfk}\cdot\frac{K}{M}\right)} \qquad (2)$$

$$=t\sqrt{2M\left[\frac{1}{m}+\frac{1}{mf}\left(\frac{F}{M}+\frac{K}{Mk}\right)\right]} \qquad (3)$$

The value, d, so defined, is the minimum difference that will be judged significant in the average case. Values of t for different probability levels are available in almost any text on statistical methods applicable to small samples. The ratios F/M and K/M can be estimated within certain limits. Equation (2) or (3) can, therefore, be used to approximate d for given values of m, f, and k. The fact that d will be given in terms of M will not impair its usefulness for comparative purposes. This is all that is necessary since the authors do not intend that the values of d determined in this way shall be used for any purpose other than that of comparing the efficiency of experimental designs varying in the values of m, f, and k.

APPROPRIATE VALUE OF t

Values of t which have a specified probability of occurrence by chance vary with the number of degrees of freedom. This raises the question of how to determine the correct value to insert in the equation when using it to determine relative efficiency of experiments of different forms. Fisher[2] states: "* * * the very same causes that produce our real error shall also contribute the materials for computing an estimate of it" and "* * * if causes of variation affect the real error in such a way as to make no contribution to our estimate, this estimate will be vitiated, and will be incapable of providing a correct statement as to the frequency with which our real error will exceed any assigned quantity * * *" It is obvious that apparent differences between lines will be affected by the accuracy with which the sires used represent the average of their lines. Thus variation between sires affects our real error and must, therefore, contribute to any valid estimate of it. It follows that a valid estimate

[2] FISHER, R. A. THE DESIGN OF EXPERIMENTS. 252 pp., illus. Edinburgh and London. 1935.

of the error of the difference must be based on the variation between the means of the progenies of different sires within each line. Hence, the associated degrees of freedom in a comparison of two lines will be two less than the sum of the number of sires used in the two lines. This is the basis on which the authors have proceeded in the calculation of minimum differences. Thus, for an experiment in which four sires are to be used in each of two lines to be compared, the degrees of freedom are six.

ESTIMATION OF VARIANCE RATIOS

Characteristics which might be considered in the type of experiment under discussion can for our purposes be conveniently grouped in three classes depending upon the source of variance among paternal half sibs. Both variance which is genetic in origin and variance arising from the same environmental sources that cause variance among full sibs will, in each of the three classes, contribute to the variance among paternal half sibs. These will be the sole sources of variance among paternal half sibs in the case of the first group of characters. This means that there is no maternal influence and that no differences between individuals result in any way from the fact that they are out of different females except as their genotypes differ because of genetic diversity of their dams. For characters of this type, which should include milk production, fertility, egg weight, wool production, growth rate or mature weight of poultry, etc., F equals M and F/M is 1 (characters such as broodiness in chickens known to be sex-linked must be excepted). Two methods are then available for estimating K/M. Data on the offspring of different females but the same male can be used to estimate K/F if there is more than one offspring from each female. Since F and M are equal, this will also be an estimate of K/M. For example, the first value in table 1 was calculated from an analysis of variance, presented in table 2, of data on the average egg weight of four pullets from each of seven hens all bred to the same cock. As the number of hens approaches infinity the mean square for hens approaches $kF+K$. The value, 1.1879 from table 2, is therefore an estimate of $kF+K$. The value, 0.6841, estimates K. Substituting,

$$1.1879=4F+0.6841,$$
$$F=0.1260, \text{ and}$$
$$\frac{0.6841}{0.1260}=5.43, \text{ an estimate of } K/F.$$

The other method of estimating K/M is based on knowledge of the sources of variance, K and M. If G is the additively genetic variance (defined to embrace four times the fraction of the variance arising from gene interactions [3] which contributes to the correlation between half sibs) in a line and E is the variance among full sibs not additively genetic, it can be shown [3] that

$$M=\tfrac{1}{4}\,G,$$
$$K=\tfrac{1}{2}\,G+E, \text{ and}$$
$$\frac{K}{M}=\frac{\tfrac{1}{2}\,G+E}{\tfrac{1}{4}\,G}=2+4E/G.$$

If, for example, G amounted to one-half of the total variance, it would be equal to E and K/M would be 6 for the character in question.

[3] WRIGHT, SEWALL. THE ANALYSIS OF VARIANCE AND THE CORRELATIONS BETWEEN RELATIVES WITH RESPECT TO DEVIATIONS FROM AN OPTIMUM. Jour. Genet. 30: 243-256. 1935.

TABLE 1.—*Values of K/F estimated from data on poultry, swine, and sheep*

[Unpublished data from the Minnesota Agricultural Experiment Station]

Class of livestock	Characteristic	Number of animals	Number of dams	K/F
Poultry	Egg weight	28	7	5.43
		30	6	1.98
		15	6	5.14
		32	8	∞
		32	8	22.48
		32	4	∞
Total or weighted average		169	38	[1] 13.97
Swine	Daily rate of gain from 50 to 200 pounds	1,257	226	[2] 5.17
Sheep	Post-weaning growth (weight increase from 105 to 165 days of age).	178	89	10.89

[1] Weighted average of K divided by weighted average of F. ∞ arises as an estimate of K/F when the estimate of $kF+K$ is less than that for K. Since F cannot actually be negative it is apparent that this circumstance has resulted from random variation. This is to be expected occasionally when small groups of data are being used. By grouping data which gave such results with other groups for which the number of offspring per dam was the same it was possible to make use of all the data and still get positive estimates of F in all cases.

[2] Calculated from intrafarrow, intrastation variance.

TABLE 2.—*Analysis of variance of the data used in obtaining the estimate of K/F in table 1*

Variance due to—	Degrees of freedom	Sum of squares	Mean square
Hens	6	7.1277	1.1879
Within hens	21	14.3660	.6841
Total	27	21.4937	

Characters of the second class, while subject to little or no direct maternal influence, are distinguished from those of the first class by the fact that there is more nongenetic variance among paternal half sibs than among full sibs. This may be partly due to residual maternal influence but is largely due to full sibs being exposed to a more uniform environment. For example, the pigs which furnished the data for the estimation of K/F presented in table 1 were farrowed over a period of 2 months in each of the different years. Climatic conditions would therefore be more uniform for pigs within any one litter than for pigs in different litters. Further, each litter was raised in a separate lot, with the result that pasture available would vary less within than between litters. This would be true as well for exposure to parasites or infections. Thus, it was observed that on a few occasions when mange appeared, it was distributed more uniformly within than between litters. For characters of this type F exceeds M. K/F can be estimated as before. K/M and F/M can be estimated from this estimate of K/F and a knowledge of the amount of genetic variance. Post-weaning growth rate of swine will be used as an example. If it is assumed that G is 32 percent of the total variance (Whatley [4] estimates between 30 and 40 percent for 180-day weight

[4] Unpublished data.

of swine, and unpublished data from this station yield an estimate of 30 percent for post-weaning growth rate)

$$M=8 \text{ percent of total variance and}$$

$$F+K=92 \text{ percent of total variance.}$$

Since

$$K/F=5 \text{ (from table 1),}$$

$$K=\tfrac{5}{6}\times 92 \text{ percent}=76.67 \text{ percent and}$$

$$F=\tfrac{1}{6}\times 92 \text{ percent}=15.33 \text{ percent.}$$

$$K/M=\frac{76.67}{8}=9.58$$

$$F/M=\frac{15.33}{8}=1.92.$$

In cases where only one offspring is raised per female as in cattle, K/F cannot be estimated as described. However, it will be noted that where k is 1, the term $\left(\frac{F}{M}+\frac{K}{Mk}\right)$ in (3) becomes $\left(\frac{F+K}{M}\right)$, which is easily estimated from knowledge of G. Using the above figures,

$$\frac{F+K}{M}=\frac{92}{8}=11.5.$$

The third class of characters, of which birth and weaning weights are typical, are those for which maternal influences cause a large amount of variance among paternal half sibs. In reality they measure characteristics of the female more than of the offspring. Thus weaning weight of pigs is more a measure of milk secretion of the sow than of inherent capacity for growth of the pigs. If measures of this kind are thought of as describing maternal characters they can be put in the first group and treated accordingly.

Table 1 contains estimates of K/F for three different characteristics in three classes of livestock. The estimate for growth rate of swine is based on enough animals to make it fairly reliable for the conditions under which the swine were raised. The separate estimates for small groups of data are presented in addition to the value for all data considered together in the case of egg weight to demonstrate the variation to which this ratio is subject when it is based on data from small numbers of animals.

CALCULATED VALUES OF D

Table 3 is presented to show how d varies with the number of males and females used in two specified situations. The value of t was used which had, for the appropriate number of degrees of freedom, a probability of 0.05 of occurring by chance.

TABLE 3.—*Differences (relative* [1]*) necessary for significance when comparing means of two lines for various values of m and f*

WHEN $F/M=2$, $K/M=10$, $k=5$

f	m						
	2	3	4	5	6	8	10
1	9.62	5.07	3.87	3.26	2.88	2.40	2.10
2	7.45	3.93	3.00	2.53	2.23	1.86	1.63
3	6.57	3.46	2.64	2.23	1.96	1.64	1.44
4	6.09	3.21	2.45	2.06	1.82	1.52	1.33
5	5.77	3.04	2.32	1.96	1.73	1.44	1.26
6	5.56	2.93	2.23	1.88	1.66	1.38	1.21
8	5.27	2.78	2.12	1.79	1.58	1.31	1.15
10	5.09	2.68	2.05	1.73	1.52	1.27	1.11
∞	4.30	2.27	1.73	1.46	1.29	1.07	.94

WHEN $\frac{F+K}{M}=19$ AND $k=1$

1	19.24	10.14	7.74	6.52	5.75	4.80	4.20
2	13.94	7.35	5.61	4.72	4.17	3.47	3.04
3	11.65	6.14	4.69	3.95	3.48	2.90	2.54
4	10.32	5.44	4.15	3.50	3.08	2.57	2.25
5	9.43	4.97	3.79	3.20	2.82	2.35	2.06
6	8.78	4.63	3.53	2.98	2.63	2.19	1.92
8	7.90	4.16	3.18	2.68	2.36	1.97	1.73
10	7.33	3.86	2.74	2.48	2.19	1.83	1.60
∞	4.30	2.27	1.73	1.46	1.29	1.07	.94

[1] $\sqrt{M}$ has been dropped from each of the tabled values.

The first value in table 3 was obtained as follows: $f=1$, $m=2$, $k=5$, $F/M=2$, $K/M=10$, and $t=4.303$ for two degrees of freedom (see (*b*) above) and $P=0.05$. Substituting in equation (3),

$$d=4.303\sqrt{2\left[\frac{1}{2}+\frac{1}{2}\left(2+\frac{10}{5}\right)\right]}\cdot\sqrt{M}=9.62\sqrt{M}.$$

The rest of the values in the first part of table 3 were obtained in the same way by substitution of the correct values of f and m. The same procedure was followed for the second part of table 3 except that k was set at 1 and $\left(\frac{F}{M}+\frac{K}{Mk}\right)$, which equals $\left(\frac{F+K}{M}\right)$ when k is 1, at 19.

Equation (3) makes it clear that whenever the term $\left(\frac{F}{M}+\frac{K}{Mk}\right)$ is constant the value of d for any specified values of m and f will be constant as well. For example, the values of d in table 3 were calculated to apply for post-weaning growth rate in swine, setting F/M at 2, K/M at 10, and k at 5. The term $\left(\frac{F}{M}+\frac{K}{Mk}\right)$ was then equal to 4. The tabled values would have been identical for a character for which F/M was 1, K/M 6, and k 2. The values in the second part of table 3 were calculated to apply to any case where the additively genetic portion of the variance is 20 percent and k is 1. Thus $\frac{F+K}{M}$ was set at 19. This table would have been the same for a situation where F/M was 1, K/M 36 (i. e., G equal to 10.5 percent of the total variance), and k 2.

Table 3 does not include cases for which but one sire is used in each line. The reason is that in such cases there are no differences between means of progenies of sires from which to estimate experimental error and, therefore, no precise method by which to determine whether an observed difference indicates a true dissimilarity between the lines or is no larger than might occur between the progenies of two sires from the same line.

Let it be assumed that pigs from 12 litters per line are to be raised for a comparison of two lines for post-weaning growth. From table 3 the differences necessary for significance if six females are bred to each of two males in each line, if four are bred to each of three males, if three are bred to each of four males, or if two are bred to each of six males are $5.56\sqrt{M}$, $3.21\sqrt{M}$, $2.64\sqrt{M}$, or $2.23\sqrt{M}$, respectively. As another example, assume that two lines of cattle fed individually are to be compared. Because of the work and equipment required only eight animals are to be fed from each line. If we assume the variance of efficiency in the feed lot is about 20 percent additively genetic, the values in the second part of table 3 can be used. Then if the eight animals are by two males, four males, or eight males, the difference necessary for significance will be $10.32\sqrt{M}$, $5.61\sqrt{M}$, or $4.80\sqrt{M}$, respectively.

DISCUSSION

Several general conclusions can be drawn from consideration of equation (2). First, whenever mf, total females used in each line, and mfk, total animals raised in each line, are constant an increase in m always decreases d. Further, when mfk is constant any increase in m or f compensated by decrease in k will decrease d, though increasing m will decrease d more than increasing f. These facts become more important with every limitation on the number of experimental animals. The requirement for a large amount of labor on each animal as in individual feeding or determination of carcass cutout value is a typical example. In such cases anything in the design of the experiment which will make the labor expended more fruitful is especially worth while.

When K/Mk is large either because of a smaller proportion of variance being additively genetic or because of a smaller value of k, the increase in efficiency resulting from the use of more sires is relatively less. The proportion of the variance of a specific character which is additively genetic may decrease as a result of either increased inbreeding of the line (or, in the case of crosses between lines, of the lines from which the parent stock is taken) or increased environmental variation. This emphasizes the importance of standardizing environment and making measurements as accurately as possible in all experiments of this type so as to minimize nongenetic variation. It is likely that in a well-conducted experiment the nongenetic portion of variance can be reduced to some extent from what it is in the average breeding herd. For example, the ratio K/F for growth in lambs (table 1) would probably have been smaller if the weight increase had been based on the average of two or three weights at the beginning and end of the 60-day period instead of on one weight at each time. However, while K/M will vary considerably depending on the characteristic in question, standardization of environment,

the inbreeding of the line or the inbreeding of lines used in producing crosses between lines, it should be remembered that the size of K/Mk affects only the degree to which d changes with f or m, not the direction in which it changes.

Table 4 has been presented for reference purposes. It contains some of the estimates of additively genetic variance that have been made up to the present time.

TABLE 4.—*Estimates of the additively genetic portion of variance*[1] *for various characteristics of dairy cattle and swine*

Class of livestock	Characteristic	Additively genetic variance
Dairy cattle	Butterfat production	[2] 0. 20–0. 25
Swine	Post-weaning rate of gain	[3]. 24
	do	[4]. 30
	180-day weight	[5]. 30–. 40
	Thickness of back-fat	[3]. 47
	Thickness of belly	[3]. 46
	Body length	[3]. 54

[1] Additively genetic variance estimated as 4 times the correlation between half sibs is equal to G as herein defined if environmental factors have not affected the correlation. Estimates made by doubling parent offspring correlations or regressions of offspring on parent, subject to the same restriction regarding environmental factors, tend to overestimate G. There is, however, reason to believe that the amount of overestimation is usually slight.

[2] LUSH, JAY L. INTRA-SIRE CORRELATIONS OR REGRESSIONS OF OFFSPRING ON DAM AS A METHOD OF ESTIMATING HERITABILITY OF CHARACTERISTICS. Amer. Soc. Anim. Prod. Proc. 33: 293–301. 1940.

[3] LUSH, JAY L. GENETIC ASPECTS OF THE DANISH SYSTEM OF PROGENY-TESTING SWINE. Iowa Agr. Expt. Sta. Res. Bul. 204, pp. [105]–196, illus. 1939.

[4] Unpublished data from this station.

[5] Information to the authors from Dr. J. A. Whatley, Jr., of the Oklahoma Agricultural Experiment Station.

The question naturally arises whether variation among the progeny of different females could not be used in the estimation of experimental error in order to increase degrees of freedom and reduce the value of t required. To do this would imply that one was accepting the progeny of the sires actually used as being representative of the progeny of all possible sires in the particular lines compared. This would be correct only when genetic variance in a line was zero, in which case the progeny of different sires would all be alike genotypically. How much genetic variance could exist before a significant loss of precision would result from this procedure would be difficult to define. It would depend in part on the number of females used and the total number of animals raised as well as on the number of sires used. Further, one would not often have accurate knowledge of the magnitude of genetic variance. It appears that the most dependable conclusions will be reached if one takes full account of the possibility of differences between the progenies of different sires.

Experimental efficiency has been defined on the basis of a comparison of two lines. This is appropriate because many experiments will compare only two lines; and, even though several lines are being compared at one time, in the final analysis, one is interested in the difference between each line and each of the others.

SUMMARY

A formula for the estimation of the difference necessary for significance (and, therefore, experimental effiicency) in comparisons of lines of breeding has been presented. The smaller the difference necessary

for significance the more reliable is the observed difference as a criterion of the true difference between the lines. Therefore the calculated values of d represent experimental efficiency.

Tables have been compiled which demonstrate for specified conditions the effect on efficiency of varying numbers of males and females used to produce experimental animals.

Various relationships between numbers of breeding animals and efficiency have been pointed out and discussed. Because these relationships operate in all situations, though in varying degrees, their application is not limited to instances in which data for the estimation of the variance ratios in the formula are available.

In the design of any experimental comparison of lines of breeding, the cost of using extra sires must be balanced against the increased efficiency to be gained from their use. It appears certain that the use of at least three or four sires in each line will always be profitable in terms of information that can be obtained from the experiment. On the other hand, increases in the number of females used will be more effective if the number of sires is also increased so that the number of females bred to each male remains small.

COMPOSITION OF THE TOPS AND ROOTS OF THE TIMOTHY PLANT AT SUCCESSIVE STAGES OF GROWTH[1]

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INTRODUCTION

The chemical composition of certain crop plants at different stages of growth and development has been the subject of several contributions from this laboratory (*5, 18, 19, 20*).[2] Those studies were undertaken for the purpose of determining the relative and absolute quantities of the structural and nonstructural components of the plant and to ascertain their various interrelationships.

The study reported here was undertaken to determine the chemical composition of the tops and roots[3] of the timothy plant at successive stages of its development. The results are calculated both on a percentage and an absolute basis. It is believed that in this way a better understanding may be obtained of the possible precursors and interrelationships of the several components of the plant.

REVIEW OF LITERATURE

There is, at present, an extensive literature on the chemical composition of grasses in general and of timothy in particular. For the most part the analytical results have been obtained by conventional methods and are reported in the usual terms, such as crude protein, crude fiber, and nitrogen-free extract. The review presented here is limited to publications of particular interest to the present study.

Sixty-three years ago (1878) Vasey and Collier (*22*) of the United States Department of Agriculture made an extensive study of the chemical composition of many grasses and forage plants. They analyzed the ash of these plants and also determined the more important organic constituents. Among the latter, they reported the percentages of oil, wax, sugars, gum and dextrin, cellulose, "amylaceous cellulose," alkali extract, and albuminoids. Collier (*3*), in his annual report to the United States Commissioner of Agriculture for the year 1879, gives additional data on the composition of American grasses.

Vasey and Richardson (*23*) in 1884 published the results of an extensive study of the chemical composition of American grasses. Their results on the percentages of the organic components of the various grasses were reported in conventional terms, such as fat, nitrogen-free extract, crude fiber, albuminoid, nonalbuminoid, and total nitrogen. They also reported on the chemical composition of 15 grasses at different stages of maturity. In analyzing timothy, they took samples at five different periods of development. They found that the percentage of ash, albuminoid, nonalbuminoid, and

[1] Received for publication October 10, 1941.

[2] Italic numbers in parentheses refer to Literature Cited, p. 544.

[3] The term "roots" as used in this paper includes the entire subterranean part of the plant, including the bulbs.

total nitrogen decreased, whereas the fat, nitrogen-free extract, and crude fiber increased, as the plant developed and matured.

Frear, Carter, Browne, Brooks, and Norris (*9*) in 1904 reported the results of a systematic analysis of timothy hay that had been cut when well past bloom. The scheme of analysis used was a modification of that proposed by Dragendorff (*6*) and by Parsons (*16*). The several fractions obtained were subjected to a systematic examination, and the residual plant material containing the hemicelluloses was hydrolyzed with 6-percent sulfuric acid. Both xylose and arabinose were identified in the hydrolysate. The lignin (lignic acid) content of the hay was found to be 9.6 percent.

Haigh (*11*) collected timothy plants at different stages of growth. The plants were separated into four parts, namely, heads, stalks with leaves, stubble, and bulbs. The results indicated that the heads increased in dry matter throughout the growth and ripening period. The nitrogen-free extract of the heads increased at a greater rate than all the other constituents. The nitrogen-free extract and crude fiber of the stalks and leaves increased during growth and ripening. The nitrogen and ether-soluble matter of the stalks and leaves increased during growth but decreased to some extent during ripening.

Hunt, Record, and Bethke (*13*) found that the protein of timothy decreased and the crude fiber increased as the plant matured. Similar results were reported by Evans and Thatcher (*8*).

Hosterman and Hall (*12*) harvested timothy at five different stages of development, beginning when the plants were nearly fully headed and ending when they were fully mature. The results for the whole hay indicated that the percentage of crude protein decreased, whereas the percentage of crude fibre increased, as the plant developed and matured.

MATERIAL AND METHODS OF ANALYSIS

The timothy plants (*Phleum pratense L.*) used in this investigation were collected from a large field at the United States Department of Agriculture Research Center at Beltsville, Md.[4] The first harvest took place in the morning of May 8. Subsequent harvests, with the exception of the last one, which occurred 4 days after the previous one, were made at weekly intervals. The plants were taken up by the roots, freed of as much soil as possible, and quickly transported to the laboratory and counted. The roots were then cut off and washed repeatedly in running water to remove any adhering soil. The tops and roots were dried at 60° C. in a large steam drier, then spread out in the laboratory, air-dried, and weighed. The air-dried tops and roots from each harvest were ground in a Wiley mill fine enough to pass through a 60-mesh sieve. Separate samples were taken for moisture and ash determinations, and the analytical data were calculated on the moisture-free and ash-free basis.

The various determinations, except those indicated below, were made as described in a previous publication (*20*).

Hot water plus 1-percent hydrochloric acid extractives.—The determination with hot water and 1-percent hydrochloric acid was carried

[4] The writers were permitted to make these collections through the kindness of T. W. Moseley, superintendent of the experiment station, Bureau of Dairy Industry, Beltsville, Md.

out as described in a previous publication (*20*) except that no attempt was made to determine separately the hot water and the 1-percent hydrochloric acid extractives.

Crude cellulose.—Crude cellulose was determined by the method of Kürschner and Hanak (*14*).

Lignin.—Lignin was determined by the method of Goss and Phillips (*10*) except that no correction for nitrogen was made. The results are reported as percentage of ash-free crude lignin.[5]

Pectic substances.—The plant material was extracted with a hot 0.5-percent ammonium oxalate solution. The pectic substances in the ammonium oxalate extract were precipitated with acidified ethanol and determined as calcium pectate by the method of Carré and Haynes (*2*). The following is a detailed description of the method used:

The weighed sample (10 or 15 gm.) was placed in a 250-cc. centrifuge bottle containing 150 cc. of aqueous 0.5-percent ammonium oxalate solution (when a 15-gm. sample was taken, 200 cc. of the ammonium oxalate solution was used). The mixture was heated to 85° C. in a water bath, the bottle was stoppered tightly with a rubber stopper, which was securely fastened with a brass holder attached to the neck of the bottle. The bottle was placed in a large water bath provided with an electric motor, immersion heaters, constant temperature control, and a device for holding and rotating endwise 10 bottles at one time, and was heated at 85° for 1.5 hours. The bottle and contents were centrifuged, the clear supernatant solution was decanted, and a few cubic centimeters of toluene was added to the extract to check any microbial decomposition. To the residue in the centrifuge bottle 100 cc. (150 cc. with a 15-gm. sample) of fresh 0.5-percent aqueous ammonium oxalate solution was added, the mixture was well shaken and centrifuged, and the clear supernatant solution was added to the first ammonium oxalate extract. The residual material was again digested for 1.5 hours at 85° with 100 cc. (150 cc. with a 15-gm. sample) of 0.5-percent aqueous ammonium oxalate solution and again washed with the ammonium oxalate solution as already described. This operation was repeated three times more, making a total of five successive digestions at 85° and five washings. The extracts and washings were combined, filtered through a Gooch crucible, and concentrated on the steam bath to a volume of 80 cc. (120 cc. with a 15-gm. sample). To the concentrated solution 3.5 volumes of 95-percent ethanol containing 5 cc. of concentrated hydrochloric acid per liter was added, and after it had stood overnight, the precipitate of crude pectin was separated with the aid of the centrifuge. The crude pectin was shaken with 80-percent ethanol containing 5 cc. of concentrated hydrochloric acid per liter [6] and centrifuged. The supernatant liquid was discarded. This operation was repeated until the alcoholic washings no longer gave a test for oxalic acid or oxalate. The pre-

[5] In determining the percentage of lignin in the roots the following modification was introduced: A 2-gm. sample was weighed out, and this was successively extracted with a 1:2 alcohol-benzene solution and boiling 1-percent hydrochloric acid solution, the procedure outlined in the paper (*10*) already referred to being followed. The extracted sample was collected on fine quantitative filter paper and after a small hole had been punctured in the paper, the plant material was washed quantitatively with water into the large test tube used for the hydrolysis with the fuming hydrochloric acid. The tube was placed on the steam bath, and the mixture was evaporated to dryness. Fuming hydrochloric acid was added to the dry residue, and the determination was completed as prescribed by Goss and Phillips (*10*).

[6] Subsequently it was found that washing the crude pectin precipitate with 80-percent ethanol containing 10 cc. of concentrated hydrochloric acid per liter resulted in a quicker removal of the oxalic acid and oxalate.

cipitate was dissolved in 50 cc. of hot water containing 2 cc. of concentrated ammonium hydroxide solution, and the solution was filtered through a Hirsch type sintered glass funnel of fine porosity. The centrifuge bottle was washed with two 50-cc. portions of hot water, the washings were poured through the Hirsch funnel previously used, and the filtrate was collected in the same beaker containing the solution of the pectic substances. The combined filtrate and washings, after cooling to room temperature, were treated with 100 cc. of $\frac{N}{10}$ (approximately) sodium hydroxide solution and allowed to stand overnight. To this alkaline solution, 50 cc. of $\frac{N}{1}$ (approximately) acetic acid and an equal volume of $\frac{M}{1}$ (approximately) calcium chloride solution were added, and the whole was boiled for 10 minutes. The gelatinous calcium pectate precipitate was collected on a weighed filter paper, washed with hot water until free of chlorides, dried at 105° and weighed. The result was calculated as percentage of calcium pectate.

RESULTS

The results obtained are recorded in tables, 1, 2, and 3. Table 1 shows the percentage composition of the timothy plant at successive stages of growth; table 2 shows the percentages of furfural yielded by several components of the plant; and table 3 gives the weights of the various constituents.

ASH

The percentage of ash in the tops increased from 8.88 in the first harvest period to 9.69 in the second. Subsequently, there was a general, although somewhat irregular, decrease as the plants grew older. The percentage of ash in the roots varied in a rather irregular manner. This was undoubtedly due to the fact that in spite of repeated washings, it was very difficult to free the roots entirely of sand and other inorganic matter.

NITROGEN AND CRUDE PROTEIN

With the exception of the plants from the last harvest period, the percentage of nitrogen and crude protein in the tops decreased generally as the plants grew older. This is in agreement with the results previously obtained by Phillips and coworkers (*18*, *19*, *20*) in their studies of wheat, barley, and oat plants at successive stages of growth. The percentage of nitrogen and crude protein in the roots decreased in the early stages of growth of the plants, reaching a minimum in the fifth harvest period, after which there was no significant change in percentage.

METHOXYL

The percentage of methoxyl in the tops increased regularly as the plants grew older. This regular increase is explained, in part at least, by the fact that in general the lignin content increases with the increase in the age of the plant. The methoxyl group is found in many substances of vegetable origin and forms an integral part of the lignin complex. There was no appreciable variation in the percentage of methoxyl in the roots of the timothy plant at successive stages of its development.

TABLE 1.—*Composition* [1] *of timothy plant at successive stages of growth*

TOPS

Harvest No.	Ash	Nitrogen	Crude protein (N×6.25)	Methoxyl	Alcohol-benzene extractives	Hot water plus 1-percent hydrochloric acid extractives	Uronic acid (as anhydrides)	Total furfural	Pentosans	Crude cellulose	Furfural in crude [2] cellulose	Lignin [3]	Nitrogen in ash-free crude [4] lignin	Methoxyl in ash-free crude lignin	Pectic substances (as calcium pectate)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	8.88	1.71	10.68	1.22	21.53	46.10	7.02	10.75	15.76	24.9	6.13	5.98	3.78	5.33	1.57
2	9.69	1.52	9.50	1.23	20.46	48.05	6.28	10.75	16.04	23.9	5.78	5.81	3.30	5.92	1.51
3	8.94	1.18	7.37	1.34	18.20	46.98	6.14	11.62	17.60	26.6	5.63	6.20	3.25	6.82	1.48
4	6.88	1.22	7.62	1.49	18.32	45.20	5.78	13.00	20.07	28.5	6.51	6.66	3.12	7.60	1.32
5	6.14	1.16	7.25	1.62	15.97	46.66	5.90	12.82	19.73	29.2	6.54	7.35	3.02	8.48	1.38
6	5.09	.89	5.56	2.11	14.06	45.22	4.88	14.37	22.76	31.4	5.97	8.52	1.86	12.24	.86
7	5.62	.86	5.37	2.22	10.69	49.84	5.24	13.80	21.65	30.4	6.69	8.47	2.20	11.98	.88
8	5.53	1.07	6.69	2.41	10.28	47.46	4.78	14.44	22.91	32.9	6.28	8.05	2.18	14.16	1.00

ROOTS

1	12.04	0.90	5.62	1.59	14.67	53.99	7.60	16.80	25.90	31.7	4.84	8.96	2.03	7.00	1.95
2	22.22	.70	4.37	1.63	16.00	54.81	7.05	19.34	30.45	28.4	4.30	8.33	1.21	------	1.62
3	12.24	.59	3.69	1.60	16.55	54.13	7.11	16.86	26.18	28.8	4.86	7.98	1.42	8.03	1.39
4	12.92	.49	3.06	1.56	14.88	58.55	5.89	15.03	23.51	26.7	4.95	6.88	1.29	9.52	1.18
5	10.27	.45	2.81	1.53	15.49	61.16	5.76	13.83	21.51	24.2	5.03	6.97	1.33	8.81	1.10
6	10.51	.45	2.81	1.48	12.08	62.71	5.62	12.64	19.53	23.7	4.87	6.99	.87	9.36	1.03
7	10.08	.50	3.12	1.42	9.88	68.87	5.18	11.90	18.42	21.9	4.71	6.34	1.35	10.06	.79
8	9.88	.57	3.56	1.68	11.26	64.20	5.45	13.83	21.63	25.1	5.22	7.98	1.48	9.75	1.17

[1] All results, except the percentage of ash, were calculated on the moisture-free and ash-free basis. The percentage of ash was calculated on the moisture-free basis.
[2] Calculated as percentage of ash-free crude cellulose.
[3] Not corrected for nitrogen.
[4] Calculated as percentage of crude lignin.

ALCOHOL-BENZENE EXTRACTIVES

The percentage of alcohol-benzene extractives in the tops generally decreased as the plants grew older. In the roots, however, the results were rather irregular.

HOT-WATER AND 1-PERCENT HYDROCHLORIC ACID EXTRACTIVES

The fraction removed from the plant by successive extraction with hot water and with hot 1-percent hydrochloric acid consisted of a heterogeneous class of compounds, such as sugars, starch, hemicelluloses, and proteins. This explains, at least in part, the irregular results obtained in the analysis of the tops and roots of the plants.

URONIC ACIDS

In general the percentages of uronic acids in the tops and roots decreased, although not always regularly, as the plants grew older. These sugar acids are components of two important plant constituents, namely, the pectic substances and the hemicelluloses. As the plants grow older, the percentage of pectic substances, particularly in lignified tissues, decreases, while the percentage of hemicelluloses generally increases. However, as the pectic substances contain a much greater percentage of these acids than the hemicelluloses, a decrease in the percentage of pectic substances, unless accompanied by a much greater increase in the percentage of hemicelluloses, would lower the percentage of uronic acids in the plant material. This explains the general trend of the percentages of uronic acids in the tops and roots of the plants.

TOTAL FURFURAL

The furfural obtained when a plant material is distilled with 12-percent hydrochloric acid is derived from the uronic acids and from the pentoses or pentosans. These two groups of subtances are found in the gums, pectins, and hemicelluloses. A pentosan fraction also occurs in close association with the cellulose. Inasmuch as these components do not yield the same percentages of furfural when distilled with 12-percent hydrochloric acid and since some of them increase while others decrease as the plant develops and grows to maturity, the percentages of total furfural in the table represent an additive effect of several factors of unequal magnitude.

The percentage of total furfural yielded by the tops generally showed an upward trend as the plant grew and developed. This increase was not consistent, however, as is evident from the results obtained from the samples of the fifth and seventh harvests. The percentage of total furfural in the roots increased at first, reaching a maximum in the sample from the second harvest period, after which, with the exception of the roots from the last harvest period, there was a gradual decrease.

PENTOSANS

The term "pentosans" in this paper does not denote, as it usually does, the percentage of total furfural calculated as pentosans. Here it represents the difference between the total furfural and furfural derived from the uronic acids, calculated as percentage of pentosans. A description of the method used for obtaining the percentage of pentosans and a full discussion of the significance of this determination are given in a previous paper (*20*).

The percentage of pentosans in the tops increased steadily until the fifth harvest period, when there was a decrease. Subsequently it increased again, although not regularly, as the plants grew older. The percentage of pentosans in the roots was rather irregular, although the general trend was downward.

CRUDE CELLULOSE AND FURFURAL FROM CRUDE CELLULOSE

The percentage of crude cellulose in the tops generally increased, although not regularly, as the plants grew older. Thus in the plants from the first harvest period the percentage of crude cellulose was 24.9, whereas in those from the last harvest period it was 32.9. The percentage of crude cellulose in the roots was irregular. From an initial percentage of 31.7 it decreased to 21.9 in the seventh harvest period, and then it increased to 25.1 in the eighth.

The percentage of furfural yielded by the crude cellulose of both tops and roots showed no significant change during the development of the plants.

CRUDE LIGNIN, NITROGEN, AND METHOXYL IN CRUDE LIGNIN

In the values for lignin recorded in table 1, no correction was made for the nitrogen in the lignin. As was pointed out by Phillips (*17*) in the determination of the lignin content of materials rich in protein, it is not possible to apply a suitable correction for the nitrogenous complexes in the lignin because of the variability in the ratio between the increase of crude lignin and the increment of nitrogen in this material.

The percentages of crude lignin, nitrogen, and methoxyl in the crude lignin of the tops show the same general trend as those previously found in studies on wheat, barley, and oat plants (*18*, *19*, *20*), that is, the percentages of crude lignin and methoxyl in the lignin increased, in the main, while the percentage of nitrogen in the crude lignin decreased as the plants grew older. The percentages of crude lignin in the roots showed no significant variation, while the percentages of methoxyl in the crude lignin showed a slight trend upward as the plants grew older. The percentages of nitrogen in the crude lignin of the roots were rather irregular.

PECTIC SUBSTANCES

Three types of pectic substances are now recognized, namely, protopectin, pectin, and pectic acid. However, the results in the last column of table 1 represent only the total or sum of these pectic complexes in the tops and roots at different stages of growth of the plants.

The percentages of pectic substances (as calcium pectate) in both the tops and roots showed a general trend downward as the plants grew older, except for the last harvest. Bennett (*1*) in studying the composition of the tops of red clover (*Trifolium pratense*) noted a slight decrease in the percentage of pectic substances as the plants grew older. In Kentucky bluegrass (*Poa pratensis*), however, he observed no such relationship.

FURFURAL YIELDED BY SEVERAL COMPONENTS OF THE TIMOTHY PLANT

Table 2 shows the percentages of furfural yielded by several components of the tops and roots of the timothy plant at successive stages of growth. In this table an attempt was made to separate the percentages of furfural yielded by several components of the tops and

roots, thus affording a more accurate picture of the development of the furfural-yielding components. The figures in the second column are taken from table 1 and are inserted here for the sake of completeness and comparison. The figures in the third column were obtained by dividing the percentages of uronic acid anhydride recorded in table 1 by 4.60, the derivation of which is explained fully in a previous paper from this laboratory (*20*). The figures in the fifth column were obtained by multiplying the percentage of crude cellulose by the percentage of furfural in the crude cellulose (using the corresponding data in table 1) and dividing the result by 100. The figures in the seventh column were obtained by subtracting the sum of the percentages in the third and fith columns from the percentage of total furfural in the second column. The figures in the last column were obtained by multiplying the percentage of furfural from the pentoses of the polyuronides (seventh column) by 1.736. (The factor 1.736 was used because xylose when distilled with 12-percent hydrochloric acid, in accordance with the usual procedure, yields only 90 percent of the theoretical quantity of furfural).

TABLE 2.—*Furfural yielded by several components of the timothy plant*

[Calculated on basis of moisture-free and ash-free plant material]

TOPS

Harvest No.	Total furfural	Furfural from—						
		Uronic acids		Crude cellulose		Pentoses of polyuronides		Pentoses of polyuronides calculated as xylose
	Percent	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*
1	10.75	1.53	14.23	1.53	14.23	7.69	71.54	13.35
2	10.75	1.37	12.74	1.38	12.84	8.00	74.42	13.89
3	11.62	1.33	11.45	1.50	12.91	8.79	75.64	15.26
4	13.00	1.26	9.69	1.86	14.31	9.88	76.00	17.15
5	12.82	1.28	9.98	1.91	14.90	9.63	75.12	16.72
6	14.37	1.06	7.38	1.87	13.01	11.44	79.61	19.86
7	13.80	1.14	8.26	2.03	14.71	10.63	77.03	18.45
8	14.44	1.04	7.20	2.07	14.33	11.33	78.47	19.67
ROOTS								
1	16.80	1.65	9.82	1.53	9.11	13.62	81.07	23.64
2	19.34	1.53	7.91	1.22	6.31	16.59	85.78	28.80
3	16.86	1.55	9.19	1.40	8.30	13.91	82.51	24.15
4	15.03	1.28	8.52	1.32	8.78	12.43	82.70	21.58
5	13.83	1.25	9.04	1.22	8.82	11.36	82.14	19.72
6	12.64	1.22	9.65	1.15	9.10	10.27	81.25	17.83
7	11.90	1.13	9.50	1.03	8.65	9.74	81.85	16.91
8	13.83	1.18	8.53	1.31	9.47	11.34	82.00	19.69

Table 2 shows that the percentages of furfural yielded by the uronic acids of the tops decreased generally as the plants grew older. Thus, the uronic acids of the tops of the young plants furnished 14.23 percent of the total furfural, while those of the more mature plants furnished only 7.20 percent. Similar results were obtained in previous studies with the barley plant (*19*). The percentages of furfural yielded by the uronic acids of the roots, calculated as percentage of the plant material, showed a regular downward trend as the plants

grew older. When these were calculated as percentage of the total furfural, however, the decrease was not particularly striking. The percentages of furfural yielded by the crude cellulose of the tops varied only slightly as the plants grew older. The percentages of furfural yielded by the crude cellulose of the tops when calculated as percentage of the total furfural ranged from 12.84 to 14.90. The percentages of furfural from the crude cellulose of the roots were rather irregular. The percentages of furfural yielded by the pentoses of the polyuronides of the tops increased, though not regularly, as the plants grew older. In the roots, the percentages of furfural yielded by the pentoses of the polyuronides increased in the early stages of the development of the plant, then it decreased, and in the last harvest period it increased again. In all stages the greatest percentage of the total furfural was furnished by the pentoses of the polyuronides. The percentages ranged from 71.54 to 85.78. The figures in the last column, of course, show the same tendency as those in the seventh column.

WEIGHTS OF PLANTS AND WEIGHTS OF VARIOUS CONSTITUENTS AT DIFFERENT STAGES OF GROWTH

Table 3 shows the weights of the tops, of the roots, and of the more important constituents at different stages in the development of the timothy plant, all calculated on the basis of 100 plants.

The weights of the moisture-free matter in the tops and roots of the plants require no special comment. There was a regular and rapid increase in the weights of the tops as the plants grew and developed. The weights of the roots also increased regularly with the increase in the age of the plants, although, of course, not at the same rate or to the same extent as the tops.

The crude protein of the tops increased as the plants developed and approached maturity. The decrease from 1.39 gm. of crude protein in the first harvest period to 0.98 gm. in the third harvest period is undoubtedly due to some error in manipulation. The increase in crude protein in the tops was nearly 4.9 times as great in the last harvest period as in the first. The quantity of crude protein in the roots showed little variation in the early stages of the development of the plants, but in the later stages there was a pronounced increase.

The methoxyl in the tops and in the roots increased regularly as the plants grew and developed. Here, also, both the absolute and the relative increase was greater in the tops than in the roots.

The quantities of the various extractives (columns 8 and 9) in both the tops and roots increased steadily, although not uniformly, with the increase in the age of the plants.

The quantities of the uronic acids in the tops and in the roots increased steadily, with only two insignificant exceptions, as the plants developed and matured.

The pentosans, crude cellulose, lignin, and pectic substances may conveniently be considered together. In the tops all these four constituents increased steadily, with one slight exception, as the plants grew older. These results are of particular significance from the standpoint of the genetic relationship of these four plant constituents. Various theories as to the possible precursors of lignin have been proposed.

TABLE 3.—*Weight of 100 timothy plants and total weight of various constituents at successive stages of growth (1939)*

[Calculated on basis of moisture-free and ash-free plant material]

TOPS

Harvest No.	Date harvested	Height of plants	Plants harvested	Weight of 100 plants (moisture-and ash-free basis)	Weight of various constituents in 100 timothy plants at successive stages of growth								
					Crude protein	Methoxyl	Alcohol-benzene extractives	Hot water plus 1-percent hydrochloric acid extractives	Uronic acids (as anhydrides)	Pentosans	Crude cellulose	Lignin	Pectic substances (as calcium pectate)
		Centimeters	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	May 8	6–33	4,400	12.99	1.39	0.16	2.80	5.99	0.91	2.05	3.23	0.78	0.20
2	May 15	13–38	5,700										
3	May 22	15–38	4,100	13.32	.98	.18	2.42	6.26	.82	2.34	3.54	.83	.20
4	May 29	19–53	3,300	20.27	1.54	.30	3.71	9.16	1.17	4.07	5.78	1.35	.27
5	June 5	21–62	2,400	30.49	2.21	.49	4.87	14.23	1.80	6.02	8.90	2.24	.42
6	June 12	26–71	1,800	41.33	2.30	.87	5.81	18.69	2.02	9.41	12.98	3.52	.36
7	June 19	29–96	1,100	57.37	3.08	1.27	6.13	28.59	3.01	12.42	17.44	4.86	.50
8	June 23	63–112	350	101.57	6.79	2.45	10.44	48.20	4.85	23.27	33.42	8.18	1.02

ROOTS

1	May 8		4,400	5.65	0.32	0.09	0.83	3.05	0.43	1.46	1.79	0.51	0.11
2	May 15		5,700	6.70	.29	.11	1.07	3.67	.47	2.04	1.90	.56	.11
3	May 22		4,100	8.50	.31	.14	1.41	4.60	.60	2.22	2.45	.68	.12
4	May 29		3,300	12.02	.37	.19	1.79	7.04	.71	2.83	3.21	.83	.14
5	June 5		2,400	14.75	.41	.23	2.28	9.02	.85	3.17	3.57	1.03	.16
6	June 12		1,800	19.25	.54	.28	2.33	12.07	1.08	3.76	4.56	1.35	.20
7	June 19		1,100	20.41	.64	.29	2.02	14.06	1.06	3.76	4.47	1.29	.16
8	June 23		350	24.92	.89	.42	2.81	16.00	1.36	5.39	6.25	1.99	.29

It has been suggested that lignin is formed by the plant from hemicelluloses (*21*), cellulose (*4*), and from pectic substances (*7*). The results presented in table 3 show definitely that lignin was not synthesized by the plant at the expense of either the pentosans, cellulose, or the pectic substances, as all these components increased as the plants grew and developed.

The pentosans, crude cellulose, lignin, and pectic substances in the roots showed a similar trend to those of the tops except, of course, that the increment in each case was considerably less.

DISCUSSION

Early in the development of many plants the cell walls undergo a change whereby the cellulose becomes hardened, or what is generally described as lignified. The change consists in the addition to the cellulose of a substance or a group of related substances designated as lignin. A knowledge of the quantity of lignin in a forage plant at different stages of its development or maturity and the possible precursors of lignin as well as the mechanism involved in its synthesis by the plant are of interest to the animal nutritionist. There is now considerable evidence to indicate that of all major plant constituents, lignin is one that is least readily digested by ruminants and nonruminants (*15*). A complete review of the various suggestions advanced concerning the nature of the parent substance and the possible mechanism involved in the synthesis of lignin by the plant was presented in a previous paper (*20*) from this laboratory and need not be repeated here in detail. Suffice it to state that among the precursors of lignin, the following have been suggested, namely, cellulose, hemicelluloses, and pectic substances. Table 3 shows that while the absolute quantity of lignin increased as the plant developed and matured, both the cellulose and the pectic substances also increased. There was no evidence whatever that lignin was synthesized by the plant at the expense of either the cellulose or the pectic substances. No direct determination of the hemicelluloses was made in this investigation for the reason that none of the available methods was found to be sufficiently precise or accurate for this purpose. However, the data on the percentages of furfural, particularly that derived from the pentoses of the polyuronides (table 2) may give indirectly an indication of the hemicelluloses present at various stages in the development of the plant. These results, it will be observed, do not support the claim that lignin is formed by the plant from hemicelluloses. The results are more in harmony with the suggestion made in a previous publication (*20*) that the plant synthesizes lignin directly from sucrose or from its hexose sugar components, namely, glucose or fructose. The possible mechanism involved in this transformation and synthesis was indicated in the paper already referred to (*20*).

SUMMARY

A study was made of the chemical composition of the tops and roots of the timothy plant at several stages of development. The quantities of the various constituents are presented on a percentage and on an absolute basis.

After an initial increase, the percentage of ash in the tops decreased as the plants grew older. The percentage of ash in the roots was rather irregular.

The percentage of nitrogen (and crude protein) in the tops decreased generally as the plants matured. The percentage of nitrogen (and crude protein) in the roots decreased in the early stages of growth of the plants and reached a minimum in the fifth harvest period, after which there was no significant change.

The quantity of nitrogen (and crude protein) in the tops increased rapidly as the plants developed and matured. The quantity in the roots showed little variation in the early stages of growth of the plants, but in the later stages the increase was somewhat more pronounced.

The percentage and absolute quantity of methoxyl in the tops increased regularly as the plants grew and developed. There was no appreciable variation in the percentage of methoxyl in the roots at successive stages in the development of the plants, although the quanquantity of methoxyl increased somewhat.

The quantities of the various extractives in the tops and roots increased steadily, although not uniformly, with the increase in the age of the plants.

The percentages of uronic acids in the tops and roots decreased generally, although not regularly, as the plants grew older. The absolute quantities of the uronic acids in the tops and roots generally increased as the plants developed and matured.

The percentage of total furfural in the tops generally showed an upward trend as the plants grew and developed. The percentage of total furfural in the roots was rather irregular.

The percentage of pentosans in the tops generally increased as the plants grew older. The percentage of pentosans in the roots was rather irregular, although the general trend was downward.

The percentage of crude cellulose in the tops generally increased, although not regularly, as the plants grew older. The percentage of crude cellulose in the roots was rather irregular.

The percentages of crude lignin and methoxyl in the lignin of the tops increased as the plant grew older. The percentage of crude lignin in the roots showed no significant variation, while the percentage of methoxyl in the crude lignin showed a slight trend upward as the plants grew older.

The percentages of pectic substances (as calcium pectate) in the tops and roots showed a general trend downward as the plants grew older.

The absolute quantities of the pentosans, crude cellulose, lignin, and pectic substances increased quite steadily as the plants grew older. There was no evidence of any kind to indicate that the plant synthesized lignin from either cellulose, pentosans, or pectic substances.

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